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A THEORY OF AUXIN ACTION INVOLVING COENZYME A

By A. C. Leopold and Frances S. Guernsey

Agricultural Experiment Station, Lafayette, Indiana*

Communicated by K. V. Thimann, September 9, 1953

Some twenty different hormones are known to occur in plants and animals, but the mechanism of hormonal action is unknown in every case except two. It has been suggested that insulin may act through its participation in the control of the hexokinase system,[13] and progesterone through its participation in the control of ATP-ase.[7] As with the many other hormones, the mechanism of action of the plant growth hormone has remained elusive.

The plant growth hormone and its synthetic counterparts, collectively termed auxins, have not been shown to participate in any specific chemical reaction by which growth may be controlled. The only reactions in which they are known to take part are ones which lead to their destruction.[4] It has been suggested that in the growth reaction two parts of the auxin molecule, the acidic side chain and the unsaturated ring nucleus, may each react with some proteinaceous materials.[6, 10] Also, the profound influences of auxins upon growth, metabolism, and differentiation have led to the suggestion that auxins may control high-energy phosphate systems in the plant,[1, 14] but no direct evidence has been found to support either of these suggestions.

The function of coenzyme A in the synthesis and metabolism of organic acids has been recently clarified.[9, 15] It has been found that coenzyme A forms an acetyl thiol-ester which is a high-energy bond, and this ester is the essential intermediate as well as the energy source for the synthesis of many plant acids. It is interesting to note that the strongest auxins have acetic acid side chains. The present study demonstrates that auxins can react with coenzyme A, and that several characteristics of the reaction suggest its involvement in the hormonal control of growth.

Enzyme preparations of ovaries from Michigan State forcing tomato flowers were tested for their activity in esterifying auxin with coenzyme

A. Of several enzyme preparations tested, mitochondrial preparations have proved to be the most active. Mitochondrial preparations from 2 g. of ovaries from tomato flowers in full bloom were prepared according to the technique of Plaut and Plaut,[12] using the particles which were centrifuged down between 6,000 and 80,000 × G, taken up in 5 ml. of 0.25 *M* sucrose. As a source of coenzyme A, a preparation of 70% purity (Nutritional Biochemicals Corporation) was used. Adenosine triphosphate as the disodium salt (Nutritional Biochemicals Corporation, 95% purity) was incorporated into the mixture as an energy source for the reaction. Unless otherwise noted, naphthaleneacetic acid was used as the auxin for the reaction and the mixture was buffered at pH 6.5 with phosphate buffer. All reagents added before incubation were dissolved in 0.25 *M* sucrose. After incubation for 20 minutes at room temperature (25°C.) the reaction was stopped by addition of 20% trichloroacetic acid. The free sulfhydryl groupings of coenzyme A were then quantitatively measured by the nitroprusside test[8] which gives a purplish color with free sulfhydryl groups. The color intensity at 546 mμ was measured using a Beckman spectrophotometer.

It was found that the addition of the enzyme to auxin (naphthaleneacetic acid), coenzyme A, and adenosine triphosphate (ATP) resulted in a quantitative disappearance of the free sulfhydryl groups. The omission of any of the four constituents prevented the reaction from occurring. The quantitative dependence of the reaction upon auxin is shown in figure 1 (*A*), in which it can be seen that the reaction was proportional to the auxin content between 4×10^{-11} and 10^{-8} *M*. Higher concentrations of the auxin inhibited the reaction. The content of free sulfhydryl groups at various concentrations of the coenzyme A preparation are shown in figure 1 (*B*). The extent of disappearance of the sulfhydryl groups during the incubation period became proportionally greater as the concentration of coenzyme A was increased. The quantitative dependence of the reaction upon ATP can be seen in figure 1 (*C*), and the dependence upon the concentration of enzyme in figure 1 (*D*). At the highest concentrations of the enzyme preparation, turbidity increased the absorbency somewhat as can be seen in figure 1 (*D*).

Lynen, *et al.*,[9] have reported that the esterification of coenzyme A with acetoacetic acid can be reversed by shifting the pH to an alkaline reaction. In our experiments the free sulfhydryl group was found to be completely regenerated by shifting the pH to 8.0 after the reaction had been completed at pH 6.5. A pH sensitivity curve of the reaction carried out in phosphate buffer from pH 4 to 10 is shown in figure 2.

If the reaction of auxin with coenzyme A is involved in the mechanism of auxin action in plant growth, one might expect that the molecular structure of the auxin might well have an influence on the reaction, for structure is

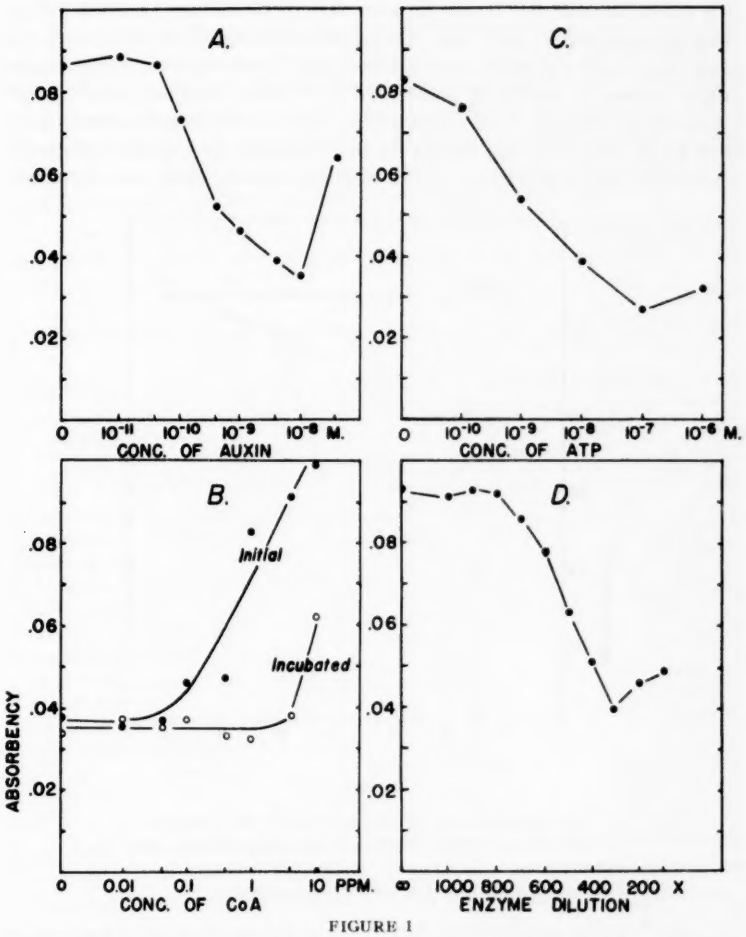

FIGURE 1

The disappearance of free sulfhydryls of coenzyme A in the presence of various concentrations of (*A*) naphthaleneacetic acid, (*B*) coenzyme A, (*C*) ATP, and (*D*) mitochondrial preparation from tomato ovaries. Except for the constituent being varied, the concentrations of each constituent were: 10^{-5} *M* naphthaleneacetic acid, 1 p.p.m. coenzyme A, 10^{-6} *M* ATP, and 1:300 enzyme. Sulfhydryls measured as absorbency at 546 mμ after the nitroprusside test.

well known to influence auxin activity in growth. A comparison of a variety of auxins and related acids as to their ability to react with coenzyme A is given in table 1. It can be seen that of the auxins tested, 2,4,5-trichlorophenoxyacetic acid and 2,4-dichlorophenoxyacetic acid were the most effective in the reaction with coenzyme A, and these are the strongest auxins known in modifying plant growth (cf. van Overbeek, *et al.*[11]). Of intermediate activity were indoleacetic acid and naphthaleneacetic acid, with lesser activity being shown for indolebutyric acid, *p*-chlorophenoxyacetic acid, indolepropionic acid, *β*-naphthoxyacetic acid, and indoleace-

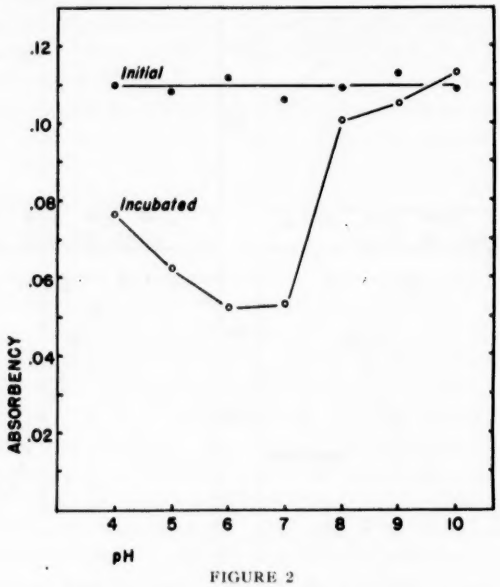

FIGURE 2

The disappearance of free sulfhydryls of coenzyme A as a function of pH. Each solution contained the complete system as in figure 1. Sulfhydryls measured by absorbency at 546 mμ after the nitroprusside test.

tonitrile. Approximately the same order of activity in the standard growth tests has been reported for these acids.[16, 18] Auxin activity has been reported for indoleacetonitrile[2] though it may be active only after conversion to the free acid.[17] Several compounds which are not auxins but are structurally closely related to auxins were also tested and found to have essentially no activity in the reaction with coenzyme A. Among these were 2,3,5-triiodobenzoic acid, 2,4-dichlorobenzoic acid, *p*-chlorobenzoic acid, and cyclohexaneacetic acid.

Reference to figure 1 (*a*) indicates that high concentrations of auxin may inhibit the reaction with coenzyme A. This may be particularly pertinent in view of the fact that while various growth phenomena in plants are stimulated by moderate concentrations of auxins, they are all inhibited by higher concentrations. A comparison of several auxins over a wide range of concentrations in the reaction with coenzyme A is shown in figure 3. It can be seen that in every instance reported auxin concentrations higher than optimum inhibited the reaction. At 10^{-6} *M* each auxin almost completely prevented the disappearance of the sulfhydryl groups.

The data presented here have shown that a mitochondrial enzyme preparation from tomato ovaries can bring about the disappearance of the

TABLE 1

COMPARISON OF THE ACTIVITY OF SEVERAL AUXINS AND RELATED ACIDS IN REACTION WITH THE SULFHYDRYL OF COENZYME A

The activity is expressed as the maximal reduction of absorbency at 546 mμ achieved by the auxin. System: enzyme at 1:300 dilution, Coenzyme A at 1 p.p.m., ATP at 10^{-6} *M*, plus acid.

ACID	RELATIVE ACTIVITY, AS MAXIMUM REDUCTION OF —SH ABSORBENCY	CONC. FOR MAXIMUM REDUCTION, *M*
2,4,5-Trichlorophenoxyacetic acid	0.057	10^{-5}
2,4-Dichlorophenoxyacetic acid	0.048	4×10^{-5}
Indoleacetic acid	0.041	4×10^{-5}
α-Naphthaleneacetic acid	0.036	10^{-5}
Indolebutyric acid	0.027	4×10^{-5}
p-Chlorophenoxyacetic acid	0.024	10^{-5}
Indolepropionic acid	0.022	4×10^{-5}
β-Naphthoxyacetic acid	0.020	10^{-5}
Indoleacetonitrile	0.018	4×10^{-5}
2,3,5-Triiodobenzoic acid	0.011	10^{-7}
2,4-Dichlorobenzoic acid	0.009	10^{-6}
p-Chlorobenzoic acid	0.009	10^{-6}
Cyclohexaneacetic acid	0.003	10^{-7}

sulfhydryl groups of coenzyme A, presumably by the formation of thiol-esters with auxins. The enzyme is present in the mitochondria where acid metabolism is chiefly carried out, and the role of auxin in growth has long been known to involve organic acid metabolism.[3] It is noteworthy that a change to alkaline conditions will restore the sulfhydryl of coenzyme A and presumably also restore free auxin. It is known that alkaline conditions may bring about the release of bound auxin from plant tissues.[5] It is highly suggestive that one form of bound auxin may be its thiol-ester with coenzyme A.

The difference in activity of the various auxins tested in this reaction with coenzyme A is approximately correlated with the activity of the auxins in inducing growth. This fact suggests that molecular structure of the

auxin, both with respect to its unsaturated nucleus and its acid side chain, markedly influences the ability of the auxin to form an ester with coenzyme A. Thus the acetic acid derivatives of the 2,4-dichlorophenoxy, indole,

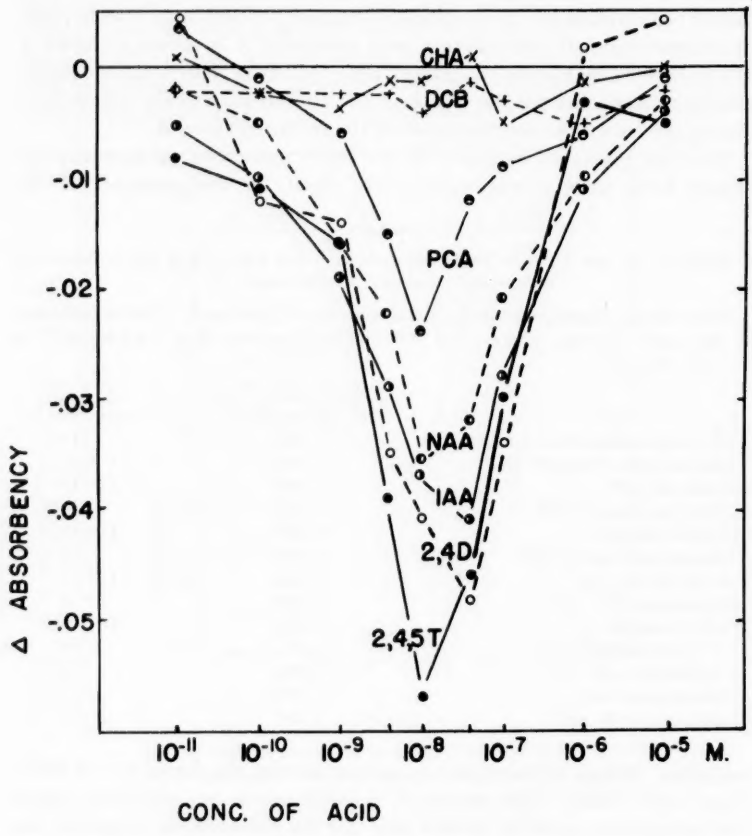

FIGURE 3

The effects of various acids on the disappearance of the free sulfhydryls of coenzyme A. Each solution contained the complete system as in figure 1 except for the auxin. 2,4,5 T = 2,4,5-trichlorophenoxyacetic acid; 2,4 D = 2,4-dichlorophenoxyacetic acid; IAA = indoleacetic acid; NAA = α-naphthaleneneacetic acid; PCA = *p*-chlorophenoxyacetic acid; DCB = 2,4-dichlorobenzoic acid; CHA = cyclohexaneacetic acid.

and cyclohexane nuclei show very different degrees of activity in the reaction with coenzyme A; and the acetic, propionic and butyric acid derivatives of the indole series likewise show different activities. It is suggested

that the molecular structure of an auxin may control its activity in growth by influencing the reaction reported here.

On the basis of these experiments it is proposed that auxin may act in plant growth and metabolism at least in part by reacting with coenzyme A. It may be assumed that the reaction product is a high-energy ester like the thiol-esters of other acids with coenzyme A. From its position as an ester with coenzyme A, it is further suggested that auxin may act to control the wide variety of synthetic and metabolic reactions in plants which are known to require coenzyme A, and that in this manner the growth hormone may regulate plant growth.

* Journal paper No. 737.

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EXPERIMENTAL COUPLING OF INDOLEACETIC ACID TO PEA ROOT PROTEIN IN VIVO AND IN VITRO

BY S. M. SIEGEL AND A. W. GALSTON

KERCKHOFF LABORATORIES OF BIOLOGY, CALIFORNIA INSTITUTE OF TECHNOLOGY

Communicated by G. W. Beadle, September 10, 1953

In attempting to understand the great biological effectiveness of low concentrations of the plant growth hormone, indoleacetic acid (IAA), plant physiologists have frequently invoked the concept that IAA, like many of the vitamins, constitutes the prosthetic group of an enzyme. According to this concept, IAA to be physiologically effective must first combine with a specific protein to form an enzyme which then catalyzes a reaction essential

to the growth process. In support of this concept, it has been shown that free auxin is released from plant tissues and plant proteins by proteolytic agents;[5, 6, 7, 10] that labeled synthetic auxins quickly find their way into substances sedimented by typical protein precipitants[2] and that the kinetics of auxin action in Avena coleoptiles are best understood in terms of a two-point attachment of auxin to some acceptor, presumably protein.[1] Despite the suggestive nature of these data, the "auxin-protein" has remained a hypothetical entity rather than a tangible reality.

In the course of work on the adaptive formation of IAA oxidase in pea roots, it was observed that proteins deposited by trichloroacetic acid (TCA) treatment of breis from roots previously incubated in 10^{-3} *M* IAA were pink, while those from control roots were white. Since TCA could be shown to yield a pink color with as little as 5 μg. of IAA on filter paper, it was reasoned that the pink color indicated protein-bound IAA. This conclusion was reinforced by treatment of the washed TCA precipitates with the Salkowski colorimetric reagent for IAA,[9] which caused the appearance of a stable deep pink color only in those proteins derived from roots previously fed IAA.

In extending this original observation, we have found that IAA is incorporated into pea root proteins in vivo by an energy-requiring process. Further, the reaction coupling IAA to protein has been successfully consummated in vitro by the use of adenosine triphosphate (ATP).

Methods.—The terminal 5 mm. of root from 2- or 3-day Alaska pea seedlings germinated at 26°C. on moist paper towels were excised and employed in experimental work within one hour after removal from the seedling. Tissue samples of 200 or 250 mg. fresh weight (*ca.* 40–50 root tips) were incubated in 50-ml. Erlenmeyer flasks in 10 ml. of *M*/60 pH 6.1 phosphate buffer, with or without added IAA. The flasks were incubated in an Aminco Dubnoff metabolic shaking incubator thermostated at 30°C. After a suitable interval, roots were recovered from the reaction vessels, rinsed with buffer, and ground in cold buffer with the aid of washed sea sand. Breis thus prepared were centrifuged at *ca.* 2000× G for 10 minutes to remove cellular debris, and the supernatant made up to a standard volume of 10 ml.

In quantitative studies of the proteins, 2 ml. aliquots of brei were treated with an equal volume of 1 *M* TCA, refrigerated one hour, then centrifuged and the supernatant decanted. The precipitates were resuspended in fresh 0.5 *M* TCA, and after standing for several minutes, recentrifuged. The precipitates were then redissolved in 1 or 2 ml. of 0.1 *N* NaOH (final pH *ca.* 11), and the Salkowski colorimetric determination of IAA carried out in the usual manner with the aid of a Klett-Summerson photoelectric colorimeter equipped with a No. 54 green filter. The proteins were analyzed for nitrogen by digestion and direct Nesslerization.[3]

Properties of the IAA-Protein.—Whether precipitated by 0.5 *M* TCA, 70% saturated ammonium sulfate or 80% aqueous acetone, the IAA-protein contained the same amount of IAA. That the IAA was firmly bound to the protein was shown by its failure to be removed by prolonged boiling, and by its persistence over the pH range 2.5 to 11.0. The IAA-protein is stable for at least 72 hours when stored at 2°C. Some of the bound IAA is released after storage for 144 hours at 2°C. or after 20 hours at 30°C.

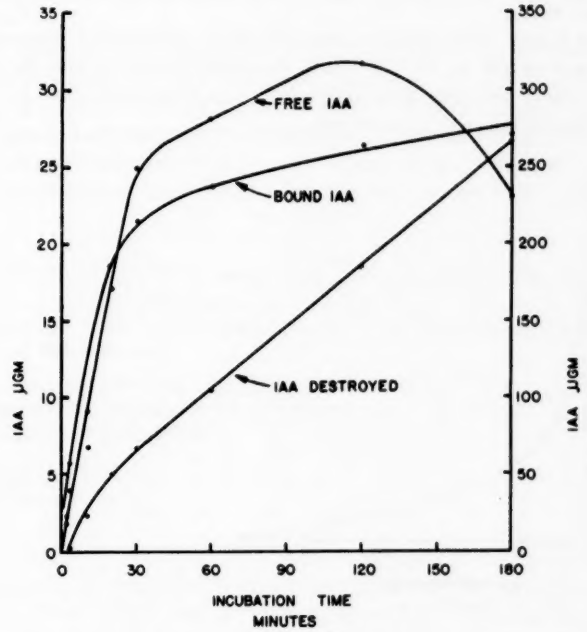


FIGURE 1

Kinetics of IAA assimilation by excised pea root tips. Left ordinate refers to curves labeled "free" and "bound IAA"; right ordinate refers to the curve labeled "IAA destroyed."

The Salkowski color of the IAA-protein is stable for at least 48–72 hours when left in contact with the reagent (which contains 50% H_2SO_4), whereas the pink color characteristic of free IAA fades in several hours to a pale orange. This is taken as further evidence of firm attachment of the IAA to the protein.

The IAA-protein was localized in the non-particulate fraction of the cell by usual centrifugation techniques.[4] Calculations of the molar ratio of IAA:protein were made for several precipitates, assuming a mean molec-

ular weight of protein of 10^5. This ratio varies from 0.1 when 10^{-4} *M* IAA is supplied to more than 1 when 10^{-3} *M* IAA is supplied.

Characteristics of the in Vivo Coupling Reaction.—TCA-precipitable IAA could be detected after one minute of incubation of the root tips with 5×10^{-4} *M* IAA. The binding of IAA to protein proceeded linearly with time up to 20 minutes of incubation, then continued at a decreased rate up to 120 minutes, approaching an asymptote at about 180 minutes. Figure 1 presents graphically the kinetics of absorption, binding and destruction of IAA fed to pea roots.

Protein-bound IAA could be detected when the external concentration of IAA was as low as 10^{-6} *M;* both the amounts bound and the ease of detection increased with increasing external IAA concentration up to 10^{-3} *M*, the highest concentration tested.

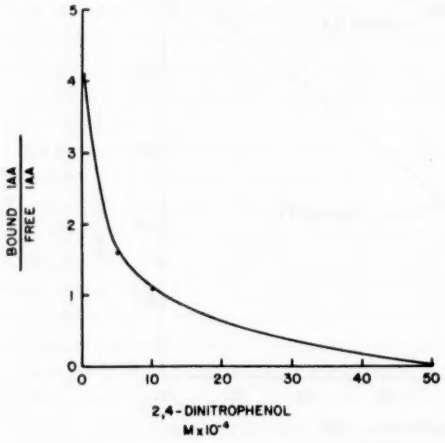


FIGURE 2

Inhibition of the in vivo coupling of IAA to pea root protein by 2,4-dinitrophenol.

The reaction coupling IAA to protein proceeds at a negligible rate at a temperature of 2°C., but rapidly at 25° or 30°C. The reaction is inhibited by 2,4-dinitrophenol (DNP), iodoacetic acid, potassium cyanide and sodium azide. These compounds at high concentrations inhibit both uptake of IAA and the binding of auxin to protein. However at lower concentrations, uptake is unaffected, but the ratio of bound:free IAA is sharply reduced, indicating that the coupling reaction itself is being interfered with. Typical inhibitional data, obtained with DNP, are shown in figure 2. Coupling of IAA to protein is also completely inhibited by 10^{-3} *M* KCN, 10^{-3} *M* NaN_3, and 5×10^{-4} *M* iodoacetic acid. Synthetic IAA analogs are also able to block the reaction, 5×10^{-4} *M* α-naphthaleneacetic acid producing a 50% inhibition.

In Vitro Studies.—Preliminary attempts to couple IAA to pea root protein by mixing protein and IAA in vitro yielded products in which IAA was much less firmly attached than in the auxin protein made in vivo. For instance, most of the IAA bound to protein in vitro was readily removed by washing of the precipitate with acetone, or by treatment with Salkowski reagent. Since the in vivo synthesis had been inhibited by various substances which interfere with the generation of energy-rich phosphate bonds, it was reasoned that the in vitro reaction might be driven by the addition of ATP.

The addition of 40 micromoles of ATP to 2 ml. of reaction mixture containing Coenzyme A, Mg^{++}, reduced glutathione, and KF was found to result in a reduction of the already low-level of IAA coupling to protein. Since Speck[8] had found a similar inhibition of glutamine synthesis to be due to excessively high levels of ATP, we reasoned that an effect of ATP might be shown if the brei were first depleted of endogenous ATP. This reduction of endogenous ATP was accomplished by aging of the brei for 16 hours at 2°C., and by preparation of an acetone powder of the pea roots.

TABLE 1

THE EFFECT OF ADDED ATP ON THE IN VITRO COUPLING OF IAA TO PEA ROOT PROTEIN

Each tube contained 1.0 ml. of the acetone powder extract, 250 μg. IAA and 40 micromoles of ATP. There were no other addenda. Autoclaved brei was treated for 15 minutes at 15 lbs. pressure.

CONDITIONS	SALKOWSKI COLOR OF REDISSOLVED TCA PRECIPITATE	PROTEIN-BOUND IAA, μG.	BOUND IAA CORRECTED, μG.
+ATP	107	13.3	8.7
−ATP	69	9.0	4.6
Autoclaved brei + ATP	30	4.6	...
Autoclaved brei − ATP	28	4.4	...

When added to aged brei, 20 micromoles of ATP stimulated the binding of IAA to protein by more than 30%. Still greater stimulations were obtained in experiments with acetone powders. Sixteen grams of freshly excised root tips from 2-day old peas were frozen on a block of solid CO_2, and were then ground in a mortar with washed sea sand and chips of solid CO_2. The frozen slurry was then exhaustively extracted with acetone previously chilled to −15°C., and the resulting material dried to a fine powder. The powder was then dissolved in $M/15$ K_2HPO_4, centrifuged free of undissolved material, made up to 25 ml. and used as a source of protein. With material prepared in this fashion, the stimulatory effect of ATP on the binding of IAA to protein was demonstrable (table 1). If correction is made for the IAA non-specifically bound to the protein in the autoclaved controls, then ATP is seen to stimulate the binding of IAA to protein by 85%.

Contrary to expectation, it was found that the coupling reaction proceeds less well in the presence of CoA than in its absence. An explanation of this anomalous situation was later supplied by the finding that the IAA-protein is rapidly cleaved into protein and TCA-soluble IAA by the addition of CoA (table 2). This cleavage appears to be specific for CoA, since other sulfhydryl-containing substances such as reduced glutathione and cysteine are, respectively, slightly active and completely inactive.

The fact that CoA removes IAA from the protein helps to explain the apparent stimulation of the in vitro coupling reaction by iodoacetate. Since iodoacetate is known to combine with sulfhydryl groups, its apparent stimulation of the coupling reaction may most probably be attributed to its combination with CoA, and thus a prevention of the reaction through which CoA removes auxin from protein. In support of this hypothesis, it was

TABLE 2

THE EFFECT OF COENZYME A AND OF OTHER SULFHYDRYL-CONTAINING REAGENTS ON THE DISSOCIATION OF IAA FROM THE IAA-PROTEIN

The IAA protein was first made by an ATP-driven in vitro reaction (60 minutes, 30°C.). Replicate tubes were then supplemented by 0.36 micromole of the sulfhydryl reagents and incubated for an additional 40 minutes. The TCA precipitates were then prepared and assayed for IAA in the usual manner.

CONDITIONS	TCA-SOLUBLE IAA, μG.	PROTEIN-BOUND IAA, μG.	TOTAL IAA, μG.
Initial	42.4	4.0	46.4
After 40 minutes			
$+H_2O$	26.0	3.5	29.5
+CoA	34.6	0	34.6
+Glutathione	30.4	2.5	32.9
+Cysteine	26.8	3.6	31.4

shown that the removal of IAA from protein in vitro by CoA is inhibited by added iodoacetate, 50% inhibition of the reaction being conferred by 5×10^{-3} M iodoacetate.

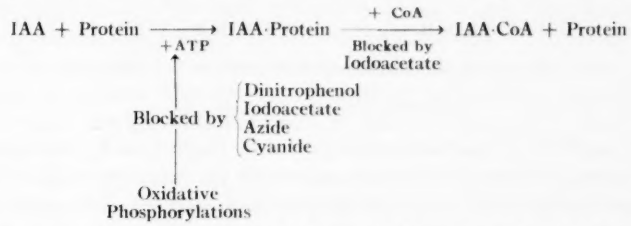
The IAA released from protein as a result of incubation with CoA was found to be largely ether insoluble at pH 3.0. Since free IAA is known to be readily soluble in ether under acid conditions, most of the IAA released from protein is not free IAA itself, but rather some water-soluble derivative. A combination of IAA with CoA or with a peptide fragment derived from the protein would satisfy these prerequisites.

Discussion.—The present work provides unambiguous confirmation of the existence of auxin-proteins in plant cells and furthermore indicates that coupling of auxin to protein is an endergonic process which can be driven by energy-rich sources such as ATP.

Whether or not the auxin-protein described here is the catalytic entity long postulated to be involved in the growth reaction, the fact that free

auxin may be firmly bound to protein must have some implications for growth. Catalytically inactive auxin-proteins may function as sequestering agents for IAA, preventing its destruction by IAA-oxidase. Alternatively, they may constitute reservoirs of IAA readily releasable and available for growth. Whatever their nature, they are of interest to the plant physiologist.

Our conception of the mechanism of the formation and cleavage of the IAA protein in vivo and in vitro may be summarized by the scheme below:



Summary.—Indoleacetic acid fed to excised pea root tips is quickly and firmly bound to protein, approximately 0.1–1.0 mole of IAA being bound per mole of protein of assumed molecular weight of 100,000. The in vivo coupling is inhibited by cyanide, azide, iodoacetate, and dinitrophenol, and is also interfered with by the presence of another auxin such as α-naphthaleneacetic acid. Auxin is not removed from the protein by dilute acid or alkali, acetone or boiling, and the auxin protein persists unchanged in vitro for at least 72 hours at 2°C.

In vitro, the coupling of auxin to protein may be accomplished with the aid of ATP. This coupling reaction is apparently inhibited by the addition of coenzyme A, which also can be shown to remove IAA in ether-insoluble form from the protein. This reaction between CoA and IAA-protein presumably involves thioether formation, since it can be prevented by the addition of iodoacetate.

Acknowledgments.—The authors wish to acknowledge a grant from the National Science Foundation which made this work possible. They also wish to thank Dr. R. S. Bandurski for advice on the in vitro experiments and for loans of ATP and CoA, and Professor James Bonner for several suggestions.

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*THE VISCOSITY AND THE CONDUCTANCE-VISCOSITY PRODUCT OF ELECTROLYTE SOLUTIONS IN BROMINE**

BY M. JOHN RICE, JR.,† AND CHARLES A. KRAUS

METCALF RESEARCH LABORATORY, BROWN UNIVERSITY

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The conductance measurements reported by Darby[1] and by Moessen and Kraus[2] for solutions of trimethylammonium chloride and tetrabutylammonium bromide in bromine indicate that these salts are highly dissociated into their ions in this solvent at higher concentrations. However, in the absence of viscosity data and in view of the apparently high viscosity of these solutions, it is not possible to draw any valid conclusion as to the extent to which ionization takes place. According to Walden's rule, the product of limiting equivalent conductance and viscosity, $\Lambda_0\eta$, has the same value for a given electrolyte in different solvents. This rule does not hold very closely for salts of small ions, particularly in water. For salts of large ions the departure from Walden's rule is not great. In solutions at higher concentrations, the conductance-viscosity product provides us with a means of approximating the extent to which a salt is ionized at different concentrations if the limiting value of the product may be evaluated for the fused salt.

We have measured the viscosity of solutions of trimethylammonium chloride and tetrabutylammonium bromide up to the highest concentration at which conductance data are available and we have extended conductance measurements for the chloride to concentrations higher than heretofore reported.

Viscosities were measured by means of viscometers of the Ostwald type, modified to meet the requirements of the material employed. They were fitted with stopcocks and ground glass caps to exclude the atmosphere and to provide for transfer of the solution from one leg of the viscometer to the other. They were calibrated with respect to water at 25° and the efflux time was obtained as a fraction of the volume of water in the viscometer so that in measuring the viscosity of different solutions it was not necessary to adjust the volume of liquid to a definite value. The amount of bromine in the viscometer was determined by weighing the viscometer and its contents.

Because of the high density and relatively high fluidity of bromine, the capillaries employed in the viscometer must have a much smaller diameter than is required with ordinary liquids. A series of viscometers was constructed having successively smaller diameters. They were calibrated against water and the efflux time with bromine was determined. The resulting viscosities as calculated in the usual way were too high until sufficiently small diameters were reached. When two viscometers of different diameters yielded the same value of the viscosity, it was assumed that the value was correct. The value so found for bromine at 25° was 0.9435 centipoise; Steacie and Johnson[3] report a value of 0.9502.

Altogether, three viscometers were employed having the flow rates of 0.0035, 0.038, and 0.20 ml./sec., respectively, with water at 25°C. The viscometers of higher flow rates were employed with solutions of higher viscosity.

Solutions were made up in the viscometer by adding known weights of salt to known weights of bromine. Suction was employed to transfer the solution from one leg to the other. The total loss of bromine after several successive transfers amounted to less than 0.02%. With increasing concentration, the loss of bromine decreased because of the marked decrease of vapor pressure of the solutions.

Conductance measurements were carried out with a cell of the Erlenmeyer type having an electrode chamber attached at one side near the bottom. The electrode chamber had a diameter of 1 cm. and the electrodes were separated by a distance of approximately 10 cm. The cell was calibrated with a 0.1 demal solution of potassium chloride. Based on the values of Jones and Bradshaw,[4] the cell constant was found to be 10.669.

Densities employed are based on values of Darby[1] and Moessen and Kraus[2] assuming that the density is a linear function of concentration. Darby has shown that the density of solutions of trimethylammonium chloride in bromine up to a concentration of 1.917 molar is a linear function of concentration. However, there is a possibility that the density function may deviate slightly from linearity at higher concentrations. This might affect viscosity values to a slight extent but not enough to affect any conclusion that may be drawn in connection with the present investigation.

The densities of the salts were determined by weighing them under hexane in a pycnometer. Assuming 0.6550 for the density of *n*-hexane, the densities of trimethylammonium chloride and tetrabutylammonium bromide were found to be 1.099 and 1.225, respectively.

The results of viscosity measurements are presented in Tables 1A and 1B for trimethylammonium chloride and tetrabutylammonium bromide, respectively. Molal concentrations (moles salt per 1000 g. bromine) are given in column 1, molar concentrations in column 2, and viscosities in centipoises in column 3.

In column 5 of Tables 2A and 2B are shown values of the conductance-viscosity product, $\Lambda\eta$, for the chloride and bromide, respectively. Molal concentrations are given in column 1, molar concentrations in column 2 and equivalent conductances in column 3. Conductance values are those of Moessen and Kraus with the exception of four values at high concentration as indicated in the table. The viscosities in column 4 were read off from large-scale plots.

Before proceeding to a discussion of the results obtained, it will be well to consider what is known about the nature of salts dissolved in bromine. Bowen and Barnes[5] have reported that tetramethylammonium bromide forms a compound with bromine which melts at about 59° and contains about 5 molecules of bromine. They further report that similar compounds are formed by tetraethylammonium bromide and tetramethylammonium chloride. The precise composition of these compounds has not been es-

TABLE 1

VISCOSITY OF SALTS IN BROMINE AT 25°

MOLAL CONC., M.	MOLAR CONC., C.	VISCOSITY, $\eta \times 10^2$	MOLAL CONC., M.	MOLAR CONC., C.	VISCOSITY $\eta \times 10^2$
A. Trimethylammonium Chloride			B. Tetrabutylammonium Bromide		
0.02132	0.06585	0.9699	0.04072	0.1234	1.121
0.1400	0.4214	1.175	0.07412	0.2186	1.257
0.2275	0.6728	1.320	0.1786	0.4898	1.641
0.3543	1.020	1.545	0.2115	0.5738	1.832
0.5121	1.425	1.912	0.2943	0.7444	2.169
0.7688	2.024	2.523	0.3600	0.8696	2.528
1.021	2.545	3.315	0.4618	1.037	3.260
1.218	2.909	4.042	0.5888	1.206	4.619
1.601	3.511	5.566	0.6663	1.292	5.923
1.994	4.000	7.104			
2.556	4.490	9.312			

tablished. Subsequent to the present investigation, Phillip L. Mercier, in this laboratory, has shown that trimethylammonium chloride forms the compound $Me_3NHCl \cdot Br_2$ which melts at 38°; there is no evidence of other compounds at temperatures above 0°. He has also shown that tetrabutylammonium bromide forms the compounds $Bu_4NBr \cdot Br_2$ and $Bu_4NBr \cdot 3Br_2$ which melt, respectively, at 75° and 37°. The two compounds form a eutectic melting at 28° with 70 mole per cent of bromine.[6] Our present results will be interpreted in the light of what we now know concerning these compounds.

In figure 1, the viscosities of solutions of each of the two salts are shown as a function of their molar concentrations. The increase of viscosity with increasing concentration is marked. At the highest concentrations measured, the viscosities of the solutions for the chloride and the bromide are, respectively, 9.9 and 6.5 times that of pure bromine. At these concentra-

tions, the solutions contain, respectively, 1.45 and 6.4 moles of free bromine, assuming that the chloride ion complexes with one and the bromide ion with three molecules of bromine.

TABLE 2

CONDUCTANCE-VISCOSITY PRODUCT FOR SALTS IN BROMINE

MOLAL CONC., M.	MOLAR CONC., C.	EQUIVALENT CONDUCTANCE, Λ	VISCOSITY, $\eta \times 10^2$	CONDUCTANCE-VISCOSITY PRODUCT, $\Lambda\eta$
	A. Trimethylammonium Chloride			
0.01168	0.03616	0.01217	0.950	0.000116
0.04050	0.1246	0.5184	1.01	0.00524
0.05182	0.1590	1.041	1.02	0.0106
0.08796	0.2678	2.863	1.08	0.0309
0.1474	0.4433	5.579	1.18	0.0658
0.2057	0.6107	7.759	1.27	0.0985
0.2855	0.8338	10.11	1.42	0.144
0.3600	1.035	11.82	1.58	0.187
0.4619	1.299	13.58	1.77	0.240
0.7623	2.011	15.75	2.50	0.394
1.058	2.617	15.29	3.45	0.528
1.549	3.436	13.01[a]	5.55	0.722
1.703	3.641	12.43	6.10	0.758
2.279	4.277	10.46[a]	8.32	0.870
2.700	4.582	9.513[a]	9.95	0.947
2.997	4.725	8.876[a]	11.00	0.976
	B. Tetrabutylammonium Bromide			
0.003836	0.01188	0.05668	0.960	0.000544
0.009556	0.02945	0.2470	0.990	0.00245
0.01803	0.05525	0.6767	1.02	0.00690
0.03032	0.09203	1.431	1.07	0.0153
0.03996	0.1206	2.060	1.12	0.0231
0.05535	0.1653	3.037	1.185	0.0360
0.05493	0.1641	2.957	1.18	0.0349
0.07510	0.2212	4.151	1.26	0.0523
0.1061	0.3056	5.828	1.37	0.0798
0.1363	0.3846	7.288	1.49	0.109
0.1632	0.4522	8.449	1.58	0.133
0.2080	0.5585	10.13	1.77	0.179
0.2406	0.6319	11.13	1.91	0.213
0.2801	0.7156	12.14	2.11	0.256
0.5326	1.135	13.92	3.90	0.543
0.6789	1.298	12.77	5.93	0.757

[a] Present investigation.

In figure 2, values of the conductance-viscosity product, $\Lambda\eta$, are plotted as a function of mole fraction of salt in solution. In computing mole fractions we have assumed that the chloride ion complexes with one molecule of bromine and the bromide ion with three molecules.

At the highest concentrations measured, the product for the chloride and the bromide has the values 0.976 and 0.757, respectively. At approx-

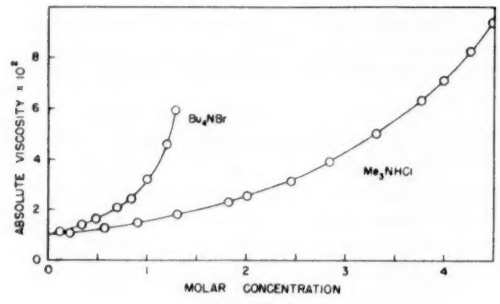

FIGURE 1

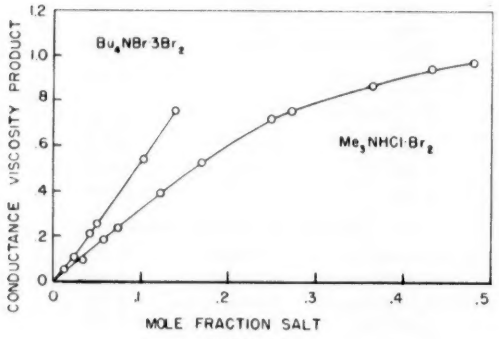

FIGURE 2

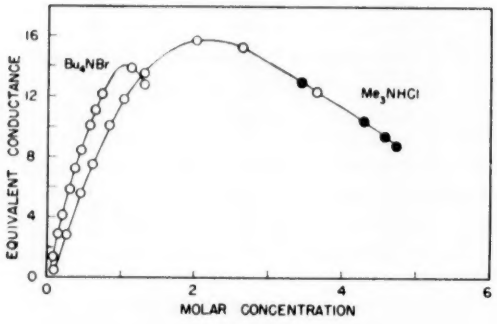

FIGURE 3

imately 0.012 molar, the product for chloride and bromide has the values 0.000116 and 0.000544, respectively. Since the viscosity of these dilute

solutions differs little from that of bromine, the change of $\Lambda\eta$ with concentration is mainly determined by change of conductance. In figure 3, the equivalent conductance of Me_3NHCl and Bu_4NBr are shown as a function of molar concentration. The maxima in the conductance curves are due to the increasing viscosity of the solutions at higher concentrations. The plots of figure 2 are, in effect, the product of the functions plotted in figures 1 and 3, except that in figure 2, mole fractions have been employed as concentration variable in place of molar concentrations, as in the other figures. If values of $\Lambda\eta$ are plotted as a function of molar concentration, the plot for Me_3NHCl is linear within the limits of experimental error; the plot for Bu_4NBr is curved strongly upward. If the value of $\Lambda_0\eta$ is a measure of the fraction of salt existing as free ions, the proportion of salt existing as ions with the chloride is proportional to the concentration of salt, i.e., the total moles of salt per unit of volume. While this conclusion is well justified by

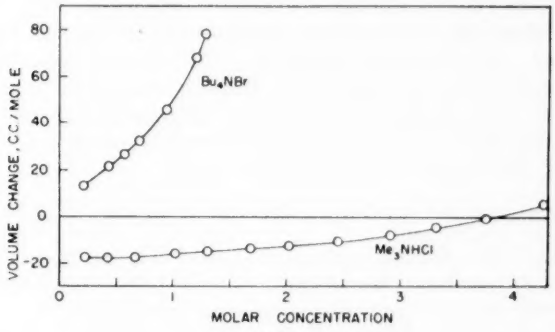

FIGURE 4

the observed results, the linear relation may, nevertheless, be fortuitous. No such simple relationship holds for tetrabutylammonium bromide. However, it should be pointed out that in the case of the chloride, the linear relation holds to the highest concentration measured where there is only 1.09 mole of bromine per mole of $Me_3NHCl \cdot Br_2$.

We have plotted values of $\Lambda\eta$ against values of mole fraction in order to gain some idea as to the value which this product might have for the pure salt. So far as the chloride is concerned, $\Lambda\eta$ appears to approach a limiting value somewhat greater than unity.

The $\Lambda\eta$ curve for Bu_4NBr is complex and the concentration is so low, 6.4 moles of bromine per mole salt, that it seems idle to speculate as to what course the curve might take at higher concentrations. However, it is of interest to note that for the bromide the plot of $\Lambda\eta$ vs. mole fraction is linear. In all likelihood, the curve for the bromide will fall off at higher

concentrations. The plot for the chloride is also linear with respect to mole fraction at lower concentrations.

Values of $\Lambda_0\eta$ enable us to approximate the fraction of salt present as ions at different concentrations. At the highest concentration, Me_3NHCl is almost completely dissociated into free ions and Bu_4NBr is highly dissociated. These solutions in bromine illustrate more clearly than do any other solutions the important fact that solutions of salts in solvents of lower dielectric constant are highly dissociated into their ions at higher concentrations. And they show, moreover, that in systems of this type, the solvent medium plays only a secondary role in determining the properties of the solution.

From the known densities of the solutions and of their components, it is possible to calculate the over-all volume change accompanying the process of solution. In figure 4 are shown values of the volume change per mole of salt for Me_3NHCl and Bu_4NBr. At the lowest concentration measured (0.227 *M*), the chloride exhibits a contraction of 13.6 ml./mol. With increasing concentration, the volume decrease diminishes and ultimately changes sign. At 4.26 *M*, the volume change is +5.1 ml./mole. The process of solution of the bromide is accompanied by an increase of volume which increases markedly with increasing concentration. At 0.218 *M*, the volume change for the bromide is 12.8 ml./mole; at 1.28 *M*, the volume change is 77.9 ml./mole.

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† This paper is based on a portion of a thesis presented by M. John Rice, Jr., in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Graduate School of Brown University, June, 1953.

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THE EFFECTS OF TEMPERATURE ON ULTRAVIOLET-INDUCED MUTABILITY IN ESCHERICHIA COLI*

BY ALEXANDER M. M. BERRIE

DEPARTMENT OF GENETICS, CARNEGIE INSTITUTION OF WASHINGTON, COLD SPRING HARBOR, NEW YORK

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The effects of ultra-violet light on *Escherichia coli* have been shown to be capable of modification by temperature[1, 2] and light.[3, 4] These effects, killing and the induction of mutations, have different responses toward the two modifying factors, heat and light. Exposure to low temperature greatly increases the lethality of ultra-violet treatment, while reducing its mutagenicity. In the case of photoreactivation, there is a similarity in the way in which "dead" and mutant bacteria respond to the reactivating light.

Witkin[1] made no special study of the lethal effect of ultra-violet light on cells of *E. coli* B/r when irradiated cells were exposed to low temperatures after treatment with *UV*, being concerned exclusively with the induction of mutants resistant to coliphage T1. Harm and Stein[2] found that the number of cells of *E. coli* B that survive irradiation with *UV* can be increased if the cells are exposed to temperatures higher than the optimal for growth. This increase in survival was found only in B, B/r showing no increase in survival at the higher temperature. Temperatures lower than 37°C. were not employed.

Demerec and Latarjet[5] made a study of the induction by *UV* and x-rays of mutants resistant to phage T1. The shape of the curves obtained when frequency of mutants was plotted against dose led these authors to suggest that the mutagenic effect of *UV* is not brought about by direct action on the genetic material but that some intermediate product, arising as a result of the irradiation and present in the cytoplasm after irradiation, effects mutation. Their x-ray data suggested that x-rays do act directly on the genetic material, since the dose-efficiency curve indicated a one-hit relationship.

In this study we have observed the dose-efficiency curves of a number of induced back mutations when the cells are exposed to different temperatures after irradiation.

Strains Employed.—Auxotrophic and streptomycin-dependent strains of *E. coli* were used. All but one, M-3, which was derived from strain B of *E. coli*, were isolated from *E. coli* B/r.

Sd-4: A strain which requires streptomycin, in addition to the normal nutrients, for growth.

12-57: An auxotroph which requires leucine for growth.

WP-14: A proline-requiring mutant.

M-3: A double auxotroph, requiring arginine and leucine.

Other amino acid-requiring strains were tested and found to be unsuitable for quantitative studies, because of the low frequency with which reversions were induced or the high degree of killing that occurred.

Methods.—Strains were grown at 37°C. in aerated nutrient broth, with the addition of 10 μg. of streptomycin per ml. in the case of Sd-4. Cultures were inoculated by seeding the broth with a small loopful of bacteria removed from the surface of a stock agar slant held at refrigerator temperature. The cultures were grown for 24 hours, by which time the cells were in a resting condition. The cells were then harvested for experimental use by centrifuging 10 ml. of the culture, decanting the supernatant, washing in 10 ml. of sterile 0.15 *M* NaCl, and resuspending in 10 ml. of saline. This suspension was diluted with saline to bring the concentration of cells to approximately 10^8 per ml., for reasons which will be explained later. The number of cells present in the suspension was estimated by the viable cell count.

Aliquots of this suspension were pipetted into flat-bottomed Petri dishes, with the depth of the suspension not greater than 2 mm.; and individual samples were irradiated at room temperature with constant agitation, so as to receive 100, 200, 300, 400, 500, 600, and 700 ergs per $mm.^2$. A General Electric Germicidal Lamp, with main emission at 2537 A, was the source of ultra-violet radiation. This lamp was calibrated by measuring its output with a General Electric Germicidal Ultraviolet Intensity Meter. All subsequent operations were carried out in a darkened room illuminated by an insect-repellent bulb, in order to prevent photoreactivation. Dilutions were made in saline at room temperature, but plating was done on plates that had been preincubated at either 37° or 15°C.

Immediately after irradiation the suspension was assayed for survivors, using the viable cell count as an estimate of the number of bacteria capable of growth. The assay platings were on nutrient-broth agar, supplemented, in the case of Sd-4, with 50 μg. of streptomycin per ml. Samples of 0.1 ml. of the irradiated suspension, or a suitable dilution, were plated onto, and spread into, the surface of an enriched synthetic medium (as used by Demerec and Cahn;[6] their medium number 3) by means of a sterile glass spreader, or onto nutrient broth agar without supplement when Sd-4 was being examined for back mutation.

In studying the response of irradiated bacteria to lowered temperature after treatment, the procedure was the same as that described; but, instead of being incubated at 37°C., the seeded plates—both those used in determining the concentration of the cell suspension and those used to estimate the induced-mutation frequency—were held at 15°C. for 30 hours and then transferred to 37°C. until the plates were ready to be scored. The plates used to determine the titer of the suspension could be scored

after 24 hours at 37°C.,[4] back mutations of the auxotrophic strains required 4 days for complete manifestation; and reversions from streptomycin dependence to non-dependence required 6 days.

In addition, a set of plates was seeded with untreated bacteria, and the number of reversions observed was used to obtain the number of spontaneously arising mutants,[5] the figures obtained from the irradiated material being corrected for this value before the frequency of induced mutants was determined. Demerec and Cahn showed that, within a wide range of inoculum size, the final number of bacteria of any particular strain populating the surface of the medium in a Petri dish containing any particular medium, is constant. This means that the number of residual divisions through which the cells pass is conditioned by the size of the inoculum. It also means that the number of spontaneous mutants arising on the plate and introduced with the inoculum will be the same for all plates, whether irradiated or non-irradiated. Sometimes it happens that during the growth of the culture from which cells are harvested for experimental use there arise mutant cells which have a selective advantage, and these increase in relative frequency to the parental type. This is seldom a serious difficulty, and the extent of such "replacement" can be found by plating out a number of different dilutions of the control suspension. The number of mutants introduced from the medium will be largest at the lowest dilutions, and smallest at the highest dilutions. It is thus very easy to calculate the background number from the numbers obtained by plating two dilutions differing by a factor of 10, and obtaining two simple simultaneous equations.

Having obtained the background figure and the number of spontaneously arising mutants (for the inoculum sizes used the background was always zero, or very low), we can correct the numbers obtained on the plates seeded with irradiated bacteria by subtracting the number of spontaneously arising mutants from them. The corrected value is recalculated on the basis of 10^8 viable cells plated.

Correction of the data obtained from the experiments with Sd-4 is carried out in a somewhat different manner. Bertani[7] showed that the cells of streptomycin-dependent strains go through a regular number of divisions when plated onto medium lacking streptomycin, regardless of the number of cells plated, except with very high inoculum sizes, which were never used in this work. The number of spontaneous mutants arising on the plates because of this residual growth is determined by the size of the inoculum. Since reversions are at a selective disadvantage in the growth medium, there is no background. In any one experiment the number of spontaneously arising reversions can be calculated by proportion for any inoculum size, if a set of plates is seeded with unirradiated cells.

Results.—Wide variation was found in the day-to-day results, and certain precautions were taken to try to eliminate as many of the variables as

possible. The suspensions that comprised the material to be irradiated never contained more than 3×10^8 cells per ml. A cell concentration of about 10^8 per ml. has been found to be the one at which no "screening" occurs; but, since we could never obtain this value consistently, some of the variation may have been due to this factor. The physiological state of the cell at irradiation has a great deal to do with its response, and since this factor differed from culture to culture it probably accounted for much of the variation.

TABLE 1

FREQUENCY OF INDUCED REVERSIONS IN Sd-4 FROM STREPTOMYCIN DEPENDENCE TO NON-DEPENDENCE AT DIFFERENT DOSES OF ULTRA-VIOLET RADIATION AND WITH POST-TREATMENT AT 15° AND 37°C.

37°C. No. of Cells/Ml. Before Treatment: 1.4×10^8

DOSE, ERGS/MM.^{2}	SURVIVORS	SURVIVING, PER CENT	NO. OF MUTANTS (6 PLATES)	NO. OF MUTANTS/10^8
100	1.09×10^8	78	729	1100
200	8.8×10^7	63	1148	2700
300	6.1×10^7	43.5	1438	3930
400	4.6×10^7	33	1495	5420
500	3.2×10^7	23	1122	5650
600	1.54×10^7	11	666	7210
700	4.0×10^6	2.86	333	13900
Control	1.4×10^8	...	11	...

15°C. No. of Cells/Ml. Before Treatment: 1.48×10^8

DOSE, ERGS/MM.^{2}	SURVIVORS	SURVIVING, PER CENT	NO. OF MUTANTS (8 PLATES)	NO. OF MUTANTS/10^8
100	6.7×10^7	45	348	620
200	4.5×10^7	30	341	860
300	3.5×10^7	24	389	1360
400	2.1×10^7	14.2	424	2500
500	1.3×10^7	8.8	365	3480
600	5.1×10^6	3.4	198[a]	4800
700	1.4×10^6	0.95	77[a]	6850
Control	1.48×10^8	...	35	...

[a] 7 and 6 plates, respectively, in treatments of 600 and 700 ergs.

Table 1 shows the results of a typical experiment in which Sd-4 was exposed to 37° and 15°C. after irradiation. The other strains used all showed the same type of response, both to increasing dose and to exposure to low temperature after treatment. Exact quantitative studies are not possible with M-3, but it behaved in a like manner.

Table 2 lists the averages of a number of experiments obtained with the three strains most exhaustively studied. The data show an increase in the frequency of induced mutants with increase in the ultra-violet dose to which cells were exposed. At the higher doses, in some of the experiments, a decrease was observed in the frequency of induced mutants per 10^8

survivors, and similar decreases have been noted by Demerec and Latarjet,[5] Stadler and Uber,[8] and Hollaender and Emmons.[9] The lowering of the frequency of induced mutants at the higher doses in this case seems to be an artifact associated with experimental difficulties, since the correction for spontaneous mutants arising during residual growth or present in the original culture no longer can be applied. In addition, the low absolute number of induced mutants recovered tends to depress the true value that would be obtained under optimal experimental conditions.

TABLE 2

AVERAGE FREQUENCY OF INDUCED REVERSIONS AND SURVIVORS IN STRAINS 12-57, SD-4, AND WP-14, WHEN SUBJECTED TO 15° AND 37°C. POST-IRRADIATION TEMPERATURE TREATMENTS

DOSE, ERGS/MM.^{2} →	100	200	300	400	500	600	700	STRAIN ↓
Survivors at 15°C., %	60.5	35.3	26.3	18.5	6.8	1.5	0.56	12-57
Survivors at 37°C., %	68.8	51.0	30.8	24.8	12.1	5.6	3.8	
Mutants/10^8 at 15°C.	32	39	53	152	528	1584	3590	
Mutants/10^8 at 37°C.	40	121	269	472	786	1342	1922	
Survivors at 15°C., %	51.0	36.0	29.0	23.3	13.3	5.5	2.7	Sd-4
Survivors at 37°C., %	65.3	47.0	35.3	29.6	18.0	8.0	2.2	
Mutants/10^8 at 15°C.	468	836	1233	1976	3030	5213	6543	
Mutants/10^8 at 37°C.	1303	2980	3803	4673	7216	8913	15900	
Survivors at 15°C., %	42.0	16.5	9.61	4.4	2.2	0.97	0.18	WP-14
Survivors at 37°C., %	59.6	35.2	19.7	10.1	3.9	1.38	0.36	
Mutants/10^8 at 15°C.	779	1573	1605	1577	4436	8440	22300	
Mutants/10^8 at 37°C.	5480	8154	10980	17930	26000	34000	31000	

When the cells were exposed to lower temperature after irradiation there was a general decrease in the frequency of induced mutants. The amount of the difference between the frequencies of induced mutants with post-treatment of 15° and 37°C. decreases at the higher doses. In one case studied here, 12–57, there were more induced mutants with the low-temperature post-treatment than with the 37°C. post-treatment when the irradiation dose was 600 or 700 ergs per mm.^{2}

Figure 1 shows the relationship between dose and frequency of induced reversion when irradiated cells of the strains employed were kept at 15° and 37°C. subsequent to treatment.

The absolute number of induced mutants varied in the manner stated above, and also with the strain. Neither of these findings was unexpected. However, although the quantitative response of the strains differed, the way in which the mutants accumulated was the same in the three strains tested.

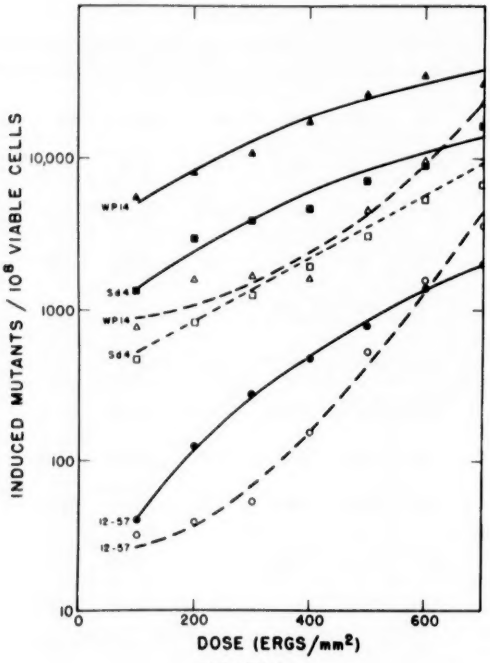

FIGURE 1

Dose-efficiency curves for induction of reversions in strains 12-57, Sd-4, and WP-14 by ultra-violet irradiation. Dotted lines: postirradiation temperature treatment 15°C. Solid lines: postirradiation temperature treatment 37°C.

Analysis of Results and Discussion.—Within the range of *UV* doses employed in these experiments, the frequency of induced mutants is proportional to the dose. The high doses, at which falling off of the mutagenic action of *UV* has been observed in Trichophyton,[9] were not used, since

the results obtained with them, for technical and statistical reasons, would not have had the same degree of validity as those obtained with the lower doses.

The number of mutant cells recovered and scored is peculiar to the genotype under investigation. However, each type of reversion follows the same qualitative pattern, such differences as exist being quantitative. Thus it is possible, by using one strain and studying one type of reversion, to investigate the process of mutagenesis initiated by *UV*.

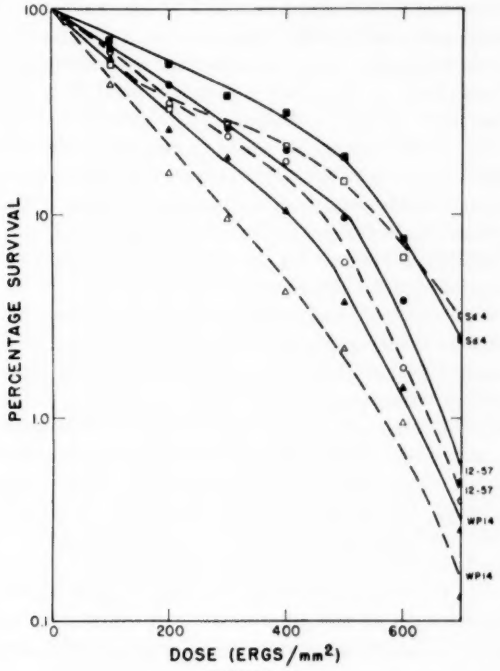

FIGURE 2

Percentage of survival at 15°C. (dotted lines) and 37°C. (solid lines) in strains 12–57, Sd–4, and WP–14, after ultra-violet irradiation.

The results show that the mutant cells are produced in all strains by similar if not identical mechanisms. Further evidence that *UV* acts generally on the cell, and not particularly on any specific genetic locus, is furnished by the pattern of increase of "dead" cells in irradiated suspensions. Among the B/r derivatives, "dead" cells accumulate with increasing doses in each strain in the same way (see Fig. 2). M-3, the B derivative, shows a different type of response to the lethal action of *UV*.

If the rate of increase of induced mutants per increase in unit dose is examined, it is seen that at 37°C. the increase is linear and the rate is a function of the strain. It appears that each gene can mutate with a finite probability determined by its own structure when it is exposed to conditions that can induce mutation. In cells exposed to 15°C. after irradiation, however, there is a different pattern of induction. In all cases the frequency of mutants increases, as the dose increases, in an exponential manner. The first type of response is that expected in a zero-order reaction, or where there is one active agent involved in the reaction. The second is associated with a reaction of higher order.

These findings substantiate the previous findings leading to the hypothesis that *UV* acts not on the genetic material itself but on some other cytoplasmic agent, or by the production in the cytoplasm of a mutagenic compound. The earlier findings referred to concerned the phenomena of photoreactivation and delayed appearance of induced mutants. The only positive and direct effect of *UV* on a physiological system that has been demonstrated so far is cessation of the synthesis of *DNA* (Kelner)[10]. This in itself would not account for the production of mutations, since the mutant cells arise some time after irradiation.

It appears that "killing" and mutation are two independent processes that *UV* is capable of accomplishing in bacterial cells. The former does not permit the cell to reproduce, whereas the latter only appears in cells that are capable of reproducing. "Killing" is reversible, in the sense that cells which have been exposed to lethal doses of *UV* can recover when exposed to light and heat. Mutation seems to be more complex, and is the result of a condition established in the cell by *UV*. Lea[11] suggested that the lethal action of *UV* results from the induction of lethal mutations. The results obtained with all the strains of *E. coli* tested have shown that low-temperature treatment is effective in reducing the frequency of induced mutants. It is not to be expected that lethal mutations would behave differently; and as the number of lethal mutants diminished the number of survivors would increase. At low temperatures there should be, if this hypothesis were correct, an increase in number of survivors. The contrary is found.

Ultra-violet light has been considered by different authors to act on the genetic material in different ways. The various hypotheses that have been advanced are: direct action; indirect action on the gene through the agency of a cytoplasmic intermediary; and the induction of a "metastable" state in the gene, from which it can go to either the parental or the mutant type.

It is conceivable that *UV* exerts a direct action on the gene; but, to account for the reversal of this effect by light or by exposure to low temperature, it must be assumed that the resulting structure is capable of reacting

to these agents. By definition the mutant structure is as stable as the original, and it is to be assumed that the mutant and the parent genes would react similarly to adverse conditions. In other words, the physiological conditions affecting treated material should affect the non-irradiated cells in some fashion. Because it is possible to alter the genetic effects of irradiation by submitting the cells to different physiological conditions after irradiation, a complex state must be assumed to have been set up in the cell as a result of irradiation.

This leads us to consider the hypotheses that mutation results indirectly from *UV* irradiation. At present the evidence from studies on photoreactivation, and the finding that exposure to low temperatures reduces the mutagenic effect of *UV*, suggest that an intermediate, photolabile reaction takes place in the cell after irradiation. Other than its reversibility, nothing is known of the nature of this reaction.

Summary.—Several auxotrophic strains and a streptomycin-dependent strain of *Escherichia coli* were used to determine the dose-efficiency curves of the mutagenic action of ultra-violet radiation. These curves were obtained for irradiated cells kept at 15° and at 37°C. after treatment. In all the strains tested, the bacteria receiving the 15° post-treatment showed a lower frequency of mutants (per 10^8 cells surviving the treatment), as well as a different pattern of increase of mutants with increasing ultra-violet doses. The dose-mutation relationship at 37° suggests a direct action of *UV* on the genetic material, but the relationship at 15° indicates a reaction of higher order. These findings are related to the currently held hypotheses concerning the mutagenic action of *UV*.

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THE EFFECTS OF INFRA-RED RADIATION AND DESICCATION ON CROSSINGOVER IN DROSOPHILA MELANOGASTER

BY P. T. IVES, B. J. FENTON, H. T. YOST, JR., AND R. P. LEVINE*

DEPARTMENT OF BIOLOGY, AMHERST COLLEGE, AMHERST, MASS.

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Previous studies have shown that following treatment with infra-red radiation, chromosomes of both Drosophila and Tradescantia are broken more frequently by subsequent treatment with x-rays.[1-4] The effects of the infra-red treatment are most pronounced in Tradescantia at a temperature of about 13°C., which is also the temperature that produces a large increase in crossingover compared to that which occurs at 25°C.[5] McElroy and Swanson[6] have advanced the view that the infra-red treatment releases an amount of energy in the chromosome insufficient to break it but which is sufficient to keep the chromosome for some time in a condition in which it is more easily broken by energy from other sources.

Crossingover has long been assumed by many geneticists to be associated normally with chromosome breakage. If this is true then somehow more chromosome breakage of this type occurs in *D. melanogaster* at 13°C. than at 25°C. One might expect, then, that treatment with infra-red radiation at 13°C. would result in a still larger amount of crossingover than occurs normally at that temperature.

This paper reports an attempt to test the above hypothesis. In the course of the experimental work unexpected evidence was obtained suggesting effects of drying on crossingover. This led to a direct test of desiccation effects, using $CaCl_2$ as a drying agent.

Materials and Methods.—The stocks used in these experiments were an Oregon-R wild-type and a second chromosome marker stock, *net b cn bw* (*net* wings, *black* body, *cinnabar* eye color, *brown* eye color). The wild-type stock had been inbred for 100 generations by single pair brother-sister matings. The marker stock was synthesized in this laboratory about ten years ago and had been maintained in close-bred vial cultures with about ten pairs of parents in each generation. At the beginning of the present study both stocks were shifted to half-pint bottle cultures and were maintained thereafter with about 100 pairs of parents per generation.

The females tested for crossingover in these studies were heterozygous for the wild-type and mutant marker stocks. They were backcrossed to *net b cn bw* immediately after experimental treatment. Individual females were mated to two or more males in shell vials and were shaken to new vials generally every third day until from three to six transfers had been made. The longer series constituted practically the entire productive period of the females.

Crossingover test vials were kept at 25°C. Flies which eclosed during the first 18 days after the parents had been admitted were scored for crossingover. They constituted practically the entire potential yields of flies. Cultures were tabulated individually for the 16 possible types of offspring, excepting in the $CaCl_2$ series where scoring was confined to crossovers in the *b-cn* region. The procedure followed made possible a statistical check of variation in crossingover and the elimination of occasional cases of aberrant results from whatever cause—non-virginity of the tested female, a chance inversion in a chromosome being tested, contamination, and so forth.

The infra-red radiation source and the method of exposing flies were similar to those reported earlier.[7] Pupae were exposed by allowing larvae to pupate on strips of towelling inserted in the medium against the vial so that the larvae pupated chiefly on one side of the paper. About 12 hours later the strip was taken out. The wet end and all larvae were removed. Then the strip was cut into two pieces each containing 20 or more pupae. The pieces were suspended each in an empty shell vial by means of the cotton stopper used to plug culture vials. One vial was placed in line for radiation in the usual manner, with the broad side of the paper containing the pupae facing the radiation source. The other vial was wrapped in a rubber cloth and placed elsewhere in the same water bath, shielded from the radiation. Other pupae developing in the original culture served as a 25°C. control for those in the 13°C. water bath. This led to three sets of heterozygous females for testing, all of whom had spent their larval period in one culture vial. In the series of tests using adult flies for treatment there were also three sets of females which had developed completely in one culture vial. Both pupae and adults were radiated for 24 hours, the pupae during the period 0 to 36 hours after the onset of the pupal period and the adult females during the period 0 to 36 hours after eclosion.

Two series were run testing females which were radiated in the pupal stage and two testing radiated adults. One series was run testing the effects of $CaCl_2$ at 25°C. only, but with six sets of females representing different lengths of time over the drying agent. This adds to a total of 18 sets of females, 8 to 12 in each set, with each female ovipositing in from 4 to 7 vials. Some 51,000 flies were scored for crossingover. Variation between females in each group proved to be random, nor was there significant variation between the two pupal or the two adult radiation series. Accordingly, all comparable data have been lumped for consideration in this report.

Experimental Results.—Table 1 shows the amount of crossingover observed in the *b-cn* region in heterozygous females raised under the indicated temperature conditions. The first series is the 25°C. control of the other two series. The second, the pupal stage series, consisted of females which spent their larval period at 25°C. and their pupal stage at 13°C. In the

third series; the young adult, the females developed at 25°C. and were kept for three days at 13°C. beginning from 0 to 12 hours after eclosion.

In the control series there is no significant variation in the amount of crossingover in the different laying periods. This is shown in the *P* value of 0.68 obtained by the use of chi-square. Whatever variation may have been caused by the increasing age of the females was statistically un-

TABLE 1

CROSSINGOVER IN SUCCESSIVE CLUTCHES OF EGGS IN THE *black-cinnabar* REGION. FEMALES KEPT IN PART AT 13°C.; AT 25°C. OTHERWISE

	LAYING PERIOD (3 DAYS EACH)						
	1	2	3	4	5	6	7
Control (at 25°C.)							
Flies	584	829	712	672	651	610	447
Crossovers	33	33	35	24	27	23	21
%	5.7	4.0	4.9	3.6	4.1	3.8	4.7
Pupal Stage at 13°C.							
Flies	924	856	607	650	324	664	63
Crossovers	91	72	39	33	17	30	2
%	9.8	8.4	6.4	5.1	5.2	4.5	3.2
Young Adult (for 3 days) at 13°C.							
Flies	585	811	759	725	637	579	363
Crossovers	28	40	54	50	25	24	15
%	4.8	4.9	7.1	6.9	3.9	4.1	4.1

TABLE 2

CROSSINGOVER IN YOUNG ADULT FEMALES EXPOSED TO INFRA-RED RADIATION FOR 24 HOURS AT 13°C.

	LAYING PERIOD IN DAYS AFTER ECLOSION OF THE FEMALES									
	2-4	5-7	8	9	10	11	12	13	14-17	18-20
Untreated										
Flies	709	807	251	636	724	462	430	613	783	652
Crossovers	26	33	12	32	39	35	20	31	38	36
%	3.7	4.1	4.8	5.0	5.4	7.6	4.7	5.1	4.9	5.5
Radiated										
Flies	709	748	465	731	704	449	420	572	655	508
Crossovers	25	24	19	36	43	26	25	33	37	27
%	3.5	3.2	4.1	4.9	6.1	5.8	6.0	5.8	5.6	5.3

noticeable in this and all other control series of this study. All told, including data not reported in this table, there were 12,102 control flies scored, among which were 541 crossovers in the *b-cn* region. This gives a crossover percentage of 4.5, which is only one-half of the amount expected according to the standard genetic map.[8] Since there was at no time significant variation from 4.5 per cent of crossingover in our control series, we have used that figure for "normal" throughout this report.

According to the findings of Plough it is expected that in the pupal stage series in table 1 there will be higher than normal crossingover in the first week's eggs and a reduced amount thereafter, approaching normal in later periods. It can be seen that this expectation is fully realized. The combination of highly inbred Oregon-R and long closebred *net b cn bw* is thus proved to be sensitive to the effects of low temperature on crossingover. The amount of crossingover was significantly above normal (P is less than 0.01) in the first three laying periods and then dropped to the normal level.

In the young adult series it was expected that the first eggs laid would show normal crossingover and that the subsequent period of above normal crossingover would be less broad than in the pupal stage series. An inspection of the data shows that again this expectation is realized. Only in the third and fourth laying periods was crossingover above the normal level (P for those two periods together is less than 0.01).

Table 2 shows the effects of infra-red radiation at 13°C. on crossingover in treated adult flies, the untreated series in this case receiving the 24 hours of low temperature but being shielded from the radiation. Because of the shortened period of exposure to cold the females were shaken to new vials each day from the 8th through the 13th day, since it was expected that the period of enhanced crossingover might be confined to one or two of those days. It can be seen that in the untreated (non-irradiated) series only on the 11th day was there a striking increase in crossingover. It looks as though part of the eggs laid on the 10th day were also affected. The value of P for those two periods together compared to the normal (4.5 based on 12,105 flies) is less than 0.01. In the radiated series the amount of crossingover was never as high as on the 11th day of the untreated series but it was more than 1.0 unit above the normal level from the 10th through the 17th days. Taken together these five laying periods have a P value of 0.0015 when compared to normal. Considering the five periods in two groups, the first one including the 10th and 11th days and the other the three later periods, the values of P compared to normal are 0.020 and 0.018, respectively, both significant differences. Because of the smaller number of flies involved, the comparison of days 12 through 17 in the two series of table 2 shows no significant difference between them, P being 0.22 in this case. However, the consistently high level of crossingover in the radiated series in the full eight-day period, 10–17, makes it seem to us that the comparison with normal is the more important one here. Therefore, we interpret this as suggesting that infra-red radiation prolongs the effectiveness of the cold treatment. Admittedly, the difference is not as clear-cut as desired and more data are needed to establish this point beyond reasonable doubt. Certainly in the untreated series crossingover is not above the control level in the 12th through 17th days, for P is up to 0.43 in that comparison.

Table 3 shows the effects of treating young pupae with infra-red radiation in a semidry condition at 13°C. The pupae were on strips of paper in vials containing no medium or liquid. At the end of the 24-hour period the strips were replaced in vials containing food and were moistened with water. Due to the influence of outdoor winter temperature on indoor relative humidity the paper strips had become very dry during the exposure period.

Comparing the results in table 3 with those of the pupal stage series in table 1 it is at once apparent that they differ remarkably in the amount of

TABLE 3

CROSSINGOVER IN FEMALES TREATED WHILE YOUNG PUPAE WITH INFRA-RED RADIATION FOR 24 HOURS AT 13°C. IN DRY CONDITION

	LAYING PERIOD IN DAYS AFTER ECLOSION OF THE FEMALES						
	2–4	5–7	8–10	11–13	14–17	18–20	21–23
				Untreated			
Flies	741	556	781	768	834	793	604
Crossovers	24	16	30	42	31	40	32
%	3.2	2.9	3.8	5.5	3.7	5.0	5.3
				Treated			
Flies	675	525	679	601	659	664	593
Crossovers	19	16	31	28	33	26	31
%	2.8	3.0	4.6	4.7	5.0	3.9	5.2

TABLE 4

CROSSINGOVER AT 25°C. IN FEMALES PARTIALLY DESICCATED BY MEANS OF $CaCl_2$ WHILE IN EARLY PUPAL DEVELOPMENT

LAYING PERIOD IN DAYS AFTER ECLOSION		NUMBER OF HOURS OVER $CaCl_2$					
		0	24	48	72	96	108
2–7	Flies	1855	1352	1201	760	660	1370
	Crossovers	73	38	39	20	18	39
	%	3.9	2.8	3.2	2.6	2.7	2.8
8–13	Flies	1387	745	946	714	662	1099
	Crossovers	62	32	49	36	23	45
	%	4.5	4.3	5.2	5.0	3.5	4.1

crossingover in the early laying periods. What is more, they are consistently lower than the 4.3 per cent observed in similar laying periods of all controls together (200 crossovers in 4623 flies). The value of P in this comparison is less than 0.01 when the four sets of data in the first two periods in table 3 are lumped. Crossingover is lower in the two earliest periods than in the two subsequent laying periods in the series of table 3; the value of P is 0.002 for that comparison. There is obviously no difference between the two series of table 3 in amount of crossingover.

It may be concluded, therefore, that under the conditions existing in the

experimental series reported in table 3 infra-red radiation had no detectable effect on crossingover, but that something, possibly excessive drying of the pupae, did depress the amount of crossingover in the first week's eggs to a level even below that normally observed in individuals kept continuously at 25°C.

Table 4 shows the results of a direct test of the effects of severe drying of the pupae on crossingover at 25°C. In this case the dry shell vial containing pupae on a paper strip was left open and was put into a mason jar over $CaCl_2$, the jar then being closed. At the indicated number of hours the jar was opened and part of the strip of pupae was cut off and placed in a vial containing food and moistened. The rest of the pupae were returned to the $CaCl_2$ jar for more dry treatment. Thus five treated series were obtained.

It was observed that very young pupae, probably less than two hours of age, were unable to withstand the drying over $CaCl_2$. But pupae older than that apparently did not suffer; indeed, some had already eclosed in the jar at 108 hours. These findings are in substantial agreement with those of an earlier study with *D. pseudoobscura*.[9]

The females of these six series were carried through six laying periods, only four of which were classed when it became obvious that they would give the desired information. Because the first two periods were the only ones to show effects and because low counts made broader variation in some sets than had previously been encountered, the data of the first two periods and of the third and fourth periods have been grouped together in two longer laying periods for ease of presentation in table 4.

It can be seen at once that in all $CaCl_2$-treated series crossingover was below normal in the first week and in the normal range in the second week Comparison of these five series, first week's laying vs. second, and first week. vs. normal, gave P less than 0.01. Neither the intensity nor the extent of the effectiveness of the desiccation seems to have been changed by increasing the length of the desiccation period. The level of crossingover in the affected period is very similar to that observed in the first two laying periods of the "naturally" dried pupae reported in table 3.

Discussion.—The results of these experiments suggest that infra-red radiation is capable of influencing the amount of crossingover which occurs in the heterochromatic regions (not necessarily in the heterochromatin) of Drosophila chromosomes. While no effects were noticeable on crossingover in the long euchromatic regions, *net-b* and *cn-bw*, both of these regions are so long to begin with that any increase in crossingover in them would be detectable only by the use of more marker genes in each region.

In this particular instance the effect of infra-red radiation appears to be not an increase in the rate of crossingover but the extending of the effective period of low temperature. At the time when low temperature by itself

normally increases crossingover there was no evidence whatever of further increase as a result of added infra-red radiation. When pupae in semidry condition were exposed to infra-red radiation at low temperature, the amount of crossingover was not affected by the radiation. While the effects of the low temperature were also suppressed in this last test, some crossingover, at least half as much as occurs at 25°C., did nevertheless occur. Were the effects of infra-red radiation such as to increase the effectiveness of the force responsible for "spontaneous" crossover breakages, one would expect to observe such effects even in this series. It may well be that the effects of both low temperature and infra-red radiation are on some other part of the still unknown process of genetic crossingover. Indeed, we see no compelling reason at present to offer these results as evidence favoring a breakage hypothesis of crossingover.

The observations regarding the effects of pupal drying on subsequent crossingover are of particular interest because of its implications in population genetics. They do not show nor suggest what happens to the chromosomes during such treatment. They suggest that something does happen which depresses crossingover below the normal level at 25°C. and which inhibits the effects of low temperature on crossingover during the drying treatment. In the pupal stage a developing fly is immobile and is accordingly at the mercy of its environment. Presumably pupation occurs in a place most satisfactory to the physiological needs of the organism at that time. In habitats with a climate similar to much of the U. S. A. a sharp change in relative humidity might occur within a few hours thereafter, either in the form of a continuously wet or an exceptionally dry period. The inference from the present observations is that such a change could determine to a significant extent the amount of genetic recombination which occurs in the germ cells during the first week of reproductive life. Genetic recombination is one of the major means by which new phenotypes are produced in natural populations. It has already been shown that naturally occurring inversions[10-13] as well as temperature, age of the female[14] and larval nutrition[15] affect the amount of genetic recombination which may be expected to occur in the heterochromatic regions in flies of natural populations. It has not previously been supposed necessary to assume that crossingover is also under the control of atmospheric relative humidity. The control of crossingover in a natural population is obviously very complex.

One other point of genetic interest remains in the data reported here. That concerns the map distance between the genes *b* and *cn*. The standard distance is 9.0 crossover units; in the present study it was only half as long. One possible explanation is that both of the stocks used here are probably free of inversions in all chromosomes. The standard map was determined largely by using Florida wild stocks. Such stocks very often carry a high

proportion of Payne inversions in the third chromosome[16, 17] which can increase crossingover in other chromosomes.[10] It should be possible to combine one or both of the Payne inversions with one of the stocks used here and see if the amount of crossingover between *b* and *cn* then approaches 9.0 per cent. But it will never be possible to ascertain how much this may have been the cause of Bridges' finding the longer map distance between the two genes.

Summary.—Experiments were carried out to test the effects of 24 hours of infra-red radiation at 13°C. on crossingover in the *black-cinnabar* (and heterochromatic) region of the second chromosome in *Drosophila melanogaster*. Within the limits of the experimental technique used, infra-red radiation increased the duration of the effects of low temperature on crossingover but did not increase the actual rate of crossingover at the time when the low temperature itself was most effective in increasing crossingover. In tests with young pupae in a semidry condition at 13°C. there was no apparent effect of infra-red radiation, and the effects of the low temperature were suppressed. Crossingover was in fact lowered beneath the normal 25°C. level and a subsequent direct test at 25°C., using $CaCl_2$ as a drying agent, gave similar crossover values. The effects of that treatment on crossingover were no more intensive or extensive at 108 hours of drying than at 24 hours of drying. Some implications of these results are discussed.

* The experiments reported here were organized and supervised by the senior author who has also prepared the manuscript. The technical work was done by Fenton. Yost directed the infra-red radiation and Levine supervised the dessication procedure. The general problem was suggested by Yost, and all have shared in its development. The data on the effects of temperature and of infra red radiation are taken from Fenton's thesis for a Degree with Honors, submitted to the Faculty of Amherst College in 1953.

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HOMOLOGY AND COHOMOLOGY OF COMPACT CONNECTED LIE GROUPS

By Armand Borel

The Institute for Advanced Study

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The homological properties of compact connected Lie groups over real coefficients are completely known;[1] the cohomology mod. p or over the integers of the classical groups, whose study was initiated by L. S. Pontrjagin and C. Ehresmann, has also been fully investigated.[2] In this note, we shall describe the cohomology ring mod. p (p prime), and also, partly, over the integers, of the quotient groups of the classical groups and (completing earlier results announced in (I)) of Spin(n), G_2, F_4. We shall add some information on the homology ring (Pontrjagin product), derived in part from general statements connecting cohomology and Pontrjagin product formulated in No. 1; No. 2 is devoted to a converse statement to the main theorems on transgression in universal bundles of (II). The detailed proofs of these results will appear elsewhere.

Notations and Definitions.—p denotes a prime number or zero, K_p a field of characteristic p, Z_p, ($p \neq 0$), the integers mod. p, Z_0 the rational numbers; $\bigwedge(x_1, \ldots, x_k)$ is the exterior algebra (over a field which the context will make precise) generated by the x_i (in the sense of Grassmann multiplication); in a graded module, Dx will be the degree of a homogeneous element x.

$H^*(X, A)$, (resp., $H_*(X, A)$), is the direct sum of the cohomology (resp., homology), groups $H^i(X, A)$, (resp., $H_i(X, A)$), of the space X with coefficients in A; the space X has no p-torsion ($p \neq 0$), if the torsion coefficients of $H^*(X, Z)$ are not divisible by p; by convention, X is always without 0-torsion.

G always denotes a compact connected Lie group, B_G is a classifying space for G, i. e., the base-space of a universal bundle E_G for G; an element $x \in H^*(G, A)$ is universally transgressive if it is transgressive in E_G, (see (II), §18, 19); $SU(n)$, (resp., $SO(n)$), unimodular unitary group in n complex (resp., real), variables, $Sp(n)$ unitary group in n quaternionic variables; $V_{n,k}$, Stiefel manifold of orthonormal k-frames in Euclidian n-space. Finally we recall, in a slightly more general form, a definition introduced in (II) §6:

DEFINITION. *Let E be an associative algebra with unit over a ring A. The elements x_i ($i \in I$, I totally ordered set), form a simple system of generators of E if E is the weak direct sum of the monogeneous submodules generated by the unit and by all the products $x_{i_1}x_{i_2}\ldots x_{i_k}$ ($i_1 < i_2 < \ldots < i_k$; $k = 1, 2, \ldots$).*

1. *The Pontrjagin Product.*[3]—Let $h: G \times G \to G$ be the map defining

the product; it induces a map h_* of $H_*(G, K_p) \otimes H_*(G, K_p)$ into $H_*(G, K_p)$ and $h_*(a \otimes b)$ is called the Pontrjagin product of a and b; this product adds the degrees, is associative, distributive, possesses a unit which spans $H_0(G, K_p)$ but, unlike the cup-product, is not always anticommutative, even for Lie groups, as an example below will show. We recall that an element $x \in H^*(G, K_p)$ of positive degree is called *primitive* if $h^*(x) = x \otimes 1 + 1 \otimes x$, h^* being the homomorphism $H^*(G, K_p) \to H^*(G, K_p) \otimes H^*(G, K_p)$ induced by h.

PROPOSITION 1. *If $H^*(G, K_p)$ has a simple system of primitive generators, (x_i), $(1 \leq i \leq m)$, then $H_*(G, K_p)$ is an anticommutative exterior algebra generated by m elements u_i, $(Du_i = Dx_i)$, and conversely.*

This is a simple consequence of the fact that h^* and h_* are dual to each other. Since a universally transgressive element is primitive ((II), Proposition 20.1), the assumption of Proposition 1 is in particular fulfilled when $H^*(G, K_p)$ has a simple system of universally transgressive generators; this happens when G has no p-torsion ((II), Prop. 7.2 and Theor. 19.1), and also, mod. 2, in some other cases, e.g., for $G = SO(n)$, ((II), Prop. 23.1), or $G = \mathrm{Spin}(n)$, $(n \leq 9)$, G_2, F_4 (see below); in particular one sees that:

If G is a classical group, $H_*(G, K_p)$ is an anticommutative exterior algebra for all p.

The degrees of the generators are given by Prop. 1 and by the results on the classical groups previously cited; e.g., for $H_*(SO(n), Z_2)$ they are equal to $1, 2, \ldots, n-1$, as was first shown by Miller, *loc. cit.*[2]

Let X be a space on which G operates; following J. Leray,[4] one can attach to each element $u \in H_s(G, K_p)$ an endomorphism ϑ_u (for the vector space structure only), of $H^*(G, K_p)$ which decreases degrees by s. The map $u \to \vartheta_u$ is a homomorphism of $H_*(G, K_p)$ into the algebra of linear endomorphisms of $H^*(X, K_p)$); if G operates on the space Y and if there is a map $f: X \to Y$ commuting with G, then ϑ_u commutes with f^*, and also acts on the spectral sequence of f; with the help of these operators one proves:

THEOREM 1. *Let Y be a space on which G operates and let $f: G \to Y$ be a map commuting with G (acting upon itself by left translations). If $H^*(G, K_p)$ has a simple system of primitive generators, then the image of f^* is a subalgebra generated by primitive elements; if moreover $p \neq 2^5$, then $H^*(Y, K_p) = N \otimes \bigwedge P'$, where f^* annihilates N and maps $\bigwedge P'$ isomorphically into $H^*(G, K_p)$.*

Applied to the particular cases where f is the inclusion of G into an overgroup, and where f is the projection of G onto a coset space, Theorem 1 generalizes a result of Leray, *loc. cit.*,[4] Note (a), as well as Prop. 21.1 and 21.2 of (II), which were extensions of theorems due to H. Samelson.

2. *A Transgression Theorem in Universal Bundles.*—By arguments

partly analogous to but simpler than those of Chap. IV in (II), one proves the following theorem, which may be considered as a converse to Theorem 19.1 and Prop. 19.1 of (II):

THEOREM 2. *If* $H^*(B_G, K_p) = K_p[y_1, \ldots, y_m]$, $(Dy_i$ *even*$)$, *then* $H^*(G, K_p) = \bigwedge(x_1, \ldots, x_m)$, $(x_i$ *universally transgressive,* $Dx_i = Dy_i - 1)$; *if* $H^*(B_G, K_2) = K_2[y_1, \ldots, y_m]$, *then* $H^*(G, K_2)$ *has a simple system of universally transgressive generators* $x_1, \ldots, x_m$ $(Dx_i = Dy_i - 1)$. *In both cases* y_i *is an image of* x_i *by transgression in* E_G.

3. *Quotient Groups of the Classical Groups.*—The groups $\mathrm{Sp}(n)$, $SU(n)$, $SO(2n)$, $SO(2n + 1)$ have cyclic centers of respective orders 2, n, 2, 1; Γ_m will denote the subgroup of order m of one of these centers. Using the known results on the classical groups, the cohomology of the cyclic groups, the spectral sequence of regular finite coverings, and the explicit determination of $\rho^*(\Gamma_m, G)$, (defined in (II), §21), one gets:

THEOREM 3. *Let* n *be a positive integer and* s *the greatest power of* 2 *dividing* n. *Then, with* $Dx = 1$, $Dx_i = i$:[6]

$$H^*(\mathrm{Sp}(n)/\Gamma_2, Z_2) \cong Z_2[x]/(x^{4s}) \otimes \bigwedge(x_3, x_7, \ldots, \hat{x}_{4s-1}, \ldots, x_{4n-1})$$

$$H^*(SO(2n)/\Gamma_2, Z_2) \cong Z_2[x]/(x^{2s}) \otimes V$$

where V *is a unitary graded algebra having a simple system of* $2n - 2$ *generators* $v_i (1 \leq i \leq 2n - 1, i \neq 2s - 1, Dv_i = i)$, *with the relations* $v_i \cdot v_i = v_{2i}$ *if* $2i \leq n - 1$. $v_i \cdot v_i = 0$ *otherwise.*

THEOREM 4. *Let* n *be a positive integer,* m *a divisor of* n, p *a prime divisor of* m, *and* s *the greatest power of* p *dividing* n. *Then (with* $Dx = 1$, $Dy = 2$, $Dx_i = i)$, *for* $p \geq 3$ *or* $p = 2$, $m \equiv 0$ *mod.* 4:[6]

$$H^*(SU(n)/\Gamma_m, Z_p) \cong Z_p[y]/(y^s) \otimes \bigwedge(x_1, x_3, \ldots, \hat{x}_{2s-1}, \ldots, x_{2n-1}),$$

and, for $p = 2$, $m \equiv 2$ *mod.* 4:

$$H^*(SU(n)/\Gamma_m, Z_2) \cong Z_2[x]/(x^{2s}) \otimes \bigwedge(x_3, x_5, \ldots, \hat{x}_{2s-1}, \ldots, x_{2n-1}).$$

For the sake of completeness, we recall that if p does not divide m, G and G/Γ_m have the same cohomology mod. p, as follows from well-known theorems on finite regular coverings.

4. *The Spinor Group.*—The group $\mathrm{Spin}(n)$, $(n \geq 3)$, is the twofold universal covering of $SO(n)$; it admits a fibering $\mathrm{Spin}(n)/T^1 = V_{n, n-2}$; knowing the cohomology of $V_{n, n-2}$, including the Sq^i (see (II), (III), or Miller[2]), one can determine its spectral sequence and obtain not only the results formulated in (I), but the more complete:

THEOREM 5. $H^*(Spin(n), Z)$ *has torsion if and only if* $n \geq 7$, *and its torsion coefficients are then all equal to* 2. *Let* $s(n)$ *be the integer such that* $2^{s(n)-1} < n \leq 2^{s(n)}$ *and put* $a(n) = 2^{s(n)} - 1$. *Then* $H^*(Spin(n), Z_2)$ *has a simple system of* $n - s(n)$ *generators* $u_i (1 \leq i < n - s(n))$, *and* u, $(Du =$

$a(n)$, and the sequence $Du_1, \ldots, Du_{n-s(n)-1}$ is obtained from the sequence 3, 4, $\ldots$, $n-1$ by erasing all powers of 2), subject to the relations:

$$\mathrm{Sq}^i u_j = \binom{Du_j}{i} u_k \text{ if } i \leq Du_j,\ i + Du_j = Du_k$$

$$\mathrm{Sq}^i u_j = 0 \text{ otherwise};\ u \cdot u = 0.$$

Mod. 2, the group $\mathrm{Spin}(n)$ shows a rather particular behavior as regards transgression in universal bundles; in fact, by use of Theorem 2 and study of the spectral sequences of the fiberings $(Spin(n), V_{n,n-2}, S_1)$ *and* $(B_{Spin(n)}, B_{SO(n)}, B_{Z_2})$ one proves:

PROPOSITION 2. *In Theorem 5, the elements $u_i (i < n - s(n))$, may be chosen to be universally transgressive, but this is the case for u if and only if $n \leq 9$.*

In particular, $H^*(Spin(n), Z_2)$ is not generated by universally transgressive elements, and $H^*(B_{Spin(n)}, Z_2)$ is not a ring of polynomials for $n \geq 10$; these facts have also repercussions on the Pontrjagin product in $H_*(Spin(n), Z_2)$ which is not anticommutative (i.e., commutative here, since we calculate mod. 2) for $n \geq 10$. I did not completely determine $H^*(B_{Spin(n)}, Z_2)$ and $H_*(Spin(n), Z_2)$ for general n; however:

THEOREM 6. $H^*(B_{Spin(10)}, Z_2) \cong Z_2[w_4, w_6, w_7, w_8, w_{10}, w_{32}]/(w_7 \cdot w_{10})$, *with $Dw_i = i$, and $(w_7 \cdot w_{10})$ being the ideal generated by the product $w_7 \cdot w_{10}$. The algebra $H_*(Spin(10), Z_2)$ has a simple system of 6 generators $u_3, u_5, u_6, u_7, u_9, u_{15}$ $(Du_i = i)$, subject to the relations: $u_i \cdot u_i = 0$ (all i), $u_i \cdot u_j = u_j \cdot u_i$ for $i < j$ $(i, j) \neq (6, 9)$, and $u_6 \cdot u_9 = u_9 \cdot u_6 + u_{15}$.*

5. The First Two Exceptional Groups.—G_2 and F_4 denote as usual the compact exceptional groups with 14 and 52 parameters; they are necessarily simply connected.

THEOREM 7. $H^*(G_2, Z)$ *is generated by 2 elements h_3, h_{11}, $(Dh_i = i)$, with relations $h_3^4 = h_{11}^2 = h_3^2 \cdot h_{11} = 0$, such that $H^*(G_2, Z)$ is the weak direct sum of the 4 infinite cyclic groups generated by 1, h_3, h_{11}, $h_3 \cdot h_{11}$ and of the 2 cyclic groups of order 2 generated by h_3^2 and h_3^3.*

This is obtained by investigation of the fiberings $G_2/S_3 = V_{7,2}$ and $Spin(7)/G_2 = S_7$, which shows moreover:

THEOREM 8. $H^*(G_2, Z_2)$ *has a simple system of universally transgressive generators x_3, x_5, x_6 $(Dx_i = i)$, satisfying:* $\mathrm{Sq}^2 x_3 = x_5$, $\mathrm{Sq}^3 x_3 = x_6$, $\mathrm{Sq}^1 x_5 = x_6$, $\mathrm{Sq}^i x_j = 0$ *otherwise;* $H^*(G_2, Z_5) = \bigwedge(y_3, y_{11})$, $(Dy_i = i)$, *with* $\mathcal{P}^1(y_3) = y_{11}$.[7]

To investigate F_4 one uses, as indicated in (I), the spectral sequences of the fiberings deduced from the inclusions $F_4 \supset Spin(9) \supset Spin(8) \supset T^4$ and $F_4 \supset Spin(9) \supset Spin(7) \supset G_2$, where $F_4/Spin(9)$ is the projective plane over the Cayley numbers; to construct these spectral sequences, one needs some of the above results and one has to know that the image of the natural homomorphism of $H^5(F_4/Spin(9), Z)$ into $H^5(F_4, Z)$ is Z_3; this in turn

follows from the two facts: (*a*) the symetric group of three objects acts faithfully on $H^8(F_4/Spin(8), Z)$, trivially on $H^8(F_4, Z)$ and commutes with the map induced by the projection; (*b*) $H^*(Spin(9), Z_3) = \Lambda(x_3, x_7, x_{11}, x_{15})$ with $\mathcal{P}^1 x_3 = x_7$, $\mathcal{P}^1 x_{11} = x_{15}$.[7] This leads to:

THEOREM 9. *$H^*(F_4, Z) = H^*(G_2 \times S_{15}, Z) \otimes U$, where U is a unitary graded ring defined by: $U^0 = U^{23} = Z$, $U^8 = U^{16} = Z_3$ with $U^8 \cdot U^8 = U^{16}$ and $U^i = 0$ otherwise. Thus $H^*(F_4, Z_2) = H^*(G_2 \times S_{15} \times S_{23}, Z_2)$ and for $p \neq 2, 3$, $H^*(F_4, Z_p) = \Lambda(x_3, x_{11}, x_{15}, x_{23})$, $(Dx_i = i)$. Moreover $H^*(F_4, Z_2)$ has a simple system of universally transgressive generators of degrees 3, 5, 6, 15, 23 and $H^*(F_4, Z_3) \cong Z_3[x]/(x^3) \otimes \Lambda(x_3, x_7, x_{11}, x_{15})$, $(Dx_i = i, Dx = 8)$.*

The torsion coefficients of $H^*(F_4, Z)$ are therefore equal to 2 or 3; the proof also gives the following partial results concerning reduced powers: (*i*) The isomorphism $H^*(F_4, Z_2) = H^*(G_2 \times S_{15} \times S_{23}, Z_2)$ is valid at least up to degree 22 for the Sq^i; (*ii*) mod. 3, $\mathcal{P}^1 x_3 = x_7$, $\mathcal{P}^1 x_{11} = x_{15}$ and x is obtained from x_7 by the Bockstein homomorphism (for suitable x, x_i); (*iii*) mod. 5 $\mathcal{P}^1 x_3 = x_{11}$, mod. 7, $\mathcal{P}^1 x_3 = x_{15}$.[7]

[1] See H. Samelson's report, *Bull. Am. Math. Soc.*, **58**, 2–37 (1952) for references.

[2] Borel, A., *Compt. rend. Acad. Sci. (Paris)*, **232**, 1628–1630 (1951); *Ann. Math.*, **57**, 115–207 (1953), Chap. III; *Comm. Math. Helv.*, **27**, 165–197 (1953), cited in the following (I), (II), (III), Borel, A., and Serre, J.-P., *Am. J. Math.*, **75**, 409–448 (1953); also, for the orthogonal group, Miller, C. E., *Ann. Math.*, **57**, 90–115 (1953).

[3] For the sake of brevity, we have stated the results in No. 1 only for Lie groups, but they are also in part valid for H-spaces with an associative product (same proofs); also, one has analogous statements over the integers, provided G, resp. G and V, have no torsion.

[4] Leray, J., (*a*) *Compt. rend. Acad. Sci. (Paris)*, **228**, 1545–1547 (1949); (*b*) *Ibid.*, 1784–1786.

[5] $H^*(G, K_p)$ is then an exterior algebra generated by elements of odd degrees; (see (II), Proposition 6.1(*b*)).

[6] As usual, $\hat{}$ over a variable means that the variable has to be omitted.

[7] For suitable universally transgressive elements; $\mathcal{P}^1$ is the reduced power operation, which, mod. p, increases degrees by $2(p-1)$; see Steenrod, N. E., PROC. NATL. ACAD. SCI., **39**, 213–223 (1953).

ASCITES TUMOR-VIRUS SYSTEM AS A BIOLOGICAL TOOL

BY HILARY KOPROWSKI, IRENA KOPROWSKA, AND ROBERT LOVE

VIRAL AND RICKETTSIAL RESEARCH, LEDERLE LABORATORIES DIVISION, AMERICAN CYANAMID COMPANY, PEARL RIVER, NEW YORK; AND PAPANICOLAOU'S CYTOLOGY LABORATORY, DEPARTMENT OF ANATOMY, CORNELL UNIVERSITY MEDICAL COLLEGE, NEW YORK, NEW YORK

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At present we have little knowledge of the actual mechanism of infection of living cells by the so-called neurotropic viruses. Progress in this field has been impeded by the complexity of the tissues of the central nervous system which has been mainly used as the medium for viral growth.

During the past three years we have studied the effect of 13 "neurotropic" viruses upon the ascites tumors of mice.[1, 2] In the course of these investigations we have succeeded in demonstrating differences in the behavior of these viruses in relation to the same ascites tumor. Four viruses failed to propagate in the tumor cells; six were found to multiply without damage to the neoplastic cells; and three (the West Nile encephalomyelitis, Mengo encephalomyelitis, and Bunyamwera) multiplied in the ascites tumor and displayed powerful oncolytic effect, ultimately destroying its cells.

Our findings suggest that the ascites tumor-virus system offers an ideal tool for the study of the multiplication of animal viruses in cells and may throw new light on the interrelationship between different viruses based on their "growth requirements." There are, for example, no detectable differences between the invasive power of eastern equine encephalomyelitis and Mengo viruses for the central nervous system of Swiss albino mice and between the multiplication rates of these viruses in murine nervous tissue; and yet cells of the Ehrlich ascites tumor failed to support the growth of eastern equine encephalomyelitis, whereas the Mengo virus multiplied in this malignant neoplasm at a higher rate than it did in the nervous tissue.[2]

The study of the specificity of "growth requirement" is being further extended by the inclusion of ascites tumors other than the Ehrlich carcinoma,[3] which, among other properties, is characterized by tetraploid modality of chromosome number[4] and increased values in DNA content of the malignant cells as compared with normal cells.[5] The use of other ascites tumors, now available[5] and characterized by a diploid modality and lower values for DNA content, may throw additional light upon the growth requirements of different viruses. Furthermore, the availability of several types of sarcomas growing in the ascitic form renders it feasible to plan experiments on the effect of viral infection, not only upon tumors of epithelial, but also of connective tissue, origin.

The ascites tumor-virus system seems also to be well suited to the study

of the interference phenomenon between viruses. Although recent work has seemed to indicate the importance of the interference phenomenon in our understanding of the mechanism of the intracellular process of multiplication of viruses,[6, 7] such studies in the field of the so-called neurotropic viruses were limited by the complexity of tissues used as the medium of viral growth. The ascites tumor offers still another opportunity for conducting experiments on the interference phenomenon in an attempt to follow the results of "dual" viral invasion of the same homogenous cellular system through daily morphological and biological observations, since samples of ascitic fluid may be readily obtained from the experimental animal by aspiration from the peritoneal cavity. On the same score, the ascites tumor-virus system might also prove to be an effective tool in evaluating the action of chemical substances which may cause inhibition of viral multiplication through interruption of the intracellular process.

Finally, it is possible that the affinity of viruses for actively growing ascites tumor cells may permit the use of this system for a quantitative study of viral multiplication, similar to that conducted so successfully with the bacteria and bacterial viruses[8] and recently applied to the living cell-influenza virus system.[9] Ascites tumor cells grown either in vivo or in vitro may be used for this purpose, and in the latter case the study may involve not only quantitative determination of the viral population, but also observations on their qualitative composition, as determined by mutants.

Since the relationship between the ascites tumor and the virus embraces not only phenomena directly related to viral multiplication but also to the oncotic process, it should be noted that the specific cellular vulnerability of the ascites tumor to infection with Bunyamwera,[10] Mengo,[2] and West Nile[2] viruses may pose interesting problems. If the multiplication of the above infectious agents in the ascites tumor induces interference with the metabolic process of the malignant cell, resulting in its ultimate destruction, a careful study of enzymatic reaction accompanying this phenomenon might be indicated. Studies on the specificity of such a reaction for a given virus in relation to a given ascites tumor may facilitate an understanding of the mechanism of selective cell destruction.

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THE DEVELOPMENT OF TRANSPLANTED FRAGMENTS OF FUNDULUS GASTRULAE

By Jane M. Oppenheimer*

Department of Biology, Bryn Mawr College

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It was reported by Luther[1] some time ago that for the egg of *Salmo* both embryonic and extra-embryonic sectors of the blastoderm accomplished considerable differentiation after transplantation at early gastrula stages to the yolk-sac epithelium of older trout fry. At later gastrula stages the capacity for differentiation became reduced in varying degrees, especially in the grafts of extra-embryonic sectors. This fact, together with results from other experiments,[2, 3] led Luther to postulate for the trout blastoderm the existence of a physiological gradient field, the potency for differentiation in any particular sector being related to the state of activity (*Aktivitätszustand*) of its constituent cells, which varies according to the age and position of the sector.

In contrast, portions of *Fundulus* germ-ring taken from positions 90° or 180° from the embryonic axis of gastrulae of varying ages failed to differentiate when isolated on the yolk-sac epithelium of gastrulae the same age as the donors, though they differentiated when implanted within embryonic regions; no difference in behavior was detected between grafts taken from the 90° and the 180° positions, and none between grafts taken from gastrulae of different ages (Oppenheimer[4]). For Fundulus, therefore, at least under the conditions of the particular experiment performed, Luther's interpretations were not confirmed.

The experiments on Fundulus, however, were not strictly comparable to those on the trout, since they involved only germ-ring rather than the larger portions of the blastoderm transplanted by Luther. It has not been clear, therefore, whether the discrepancies in result between the two sets of experiments were caused by differences in the biological material *per se* or by differences in the experimental method. Accordingly, the experiments on Fundulus have now been supplemented by a small number of transplantations involving larger parts of the blastoderm, more closely comparable to the sectors used by Luther for his grafts. In the experimental series described in this communication, the development of sixths and quarters of the Fundulus blastoderm has been studied, and such fragments from embryonic and extra-embryonic regions have been implanted either into embryonic or extra-embryonic areas of host gastrulae. The results obtained from implantation of extra-embryonic fragments into extra-embryonic regions were dissimilar both to the earlier results on Fundulus and to those on Salmo.

The operations forming the basis of the present report were performed by grafting techniques already described[4] and were carried out under aseptic precautions. The grafts consisted either of approximate sixths (Figure 1 (A)) or quarters (Fig. 1 (B)) of gastrulae of stage 12 to 12+ and were implanted into hosts of stage 12 to 13. The number of successful cases was too low to allow distinction between the behavior of quarters and sixths. A total of 48 grafts was made, of which 37 were studied histologically in section; only the latter will be discussed here.

The grafts may be considered in four groups. Six of the grafts containing half of the embryonic shield became situated within the embryo of the host and these differentiated embryonic structures. Eight of the grafts from extra-embryonic areas became located within the host embryo; seven of these formed embryonic structures, only one remaining undifferentiated. Six grafts containing shield material developed on the yolk-sac epithelium at some distance from the host embryo; three of these differentiated embryonic structures, the other three failed to do so. These results, which are on the whole compatible with those previously reported for Fundulus, demonstrate that grafts from extra-embryonic regions are under some conditions capable of undergoing histogenesis, and that the yolk-sac epithelium is able to support differentiation.

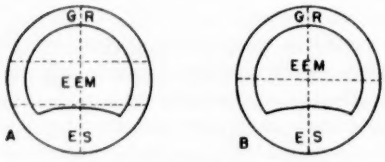

FIGURE 1

Scheme of operations. The blastoderm as seen from the animal pole of the egg consists of a central pavement-epithelium, the extra-embryonic membrane (EEM), surrounded by a ring of thicker tissue which at the embryonic pole is the embryonic shield (ES) and elsewhere the germ ring (GR). The broken lines represent the planes along which the blastoderm was divided. The fragments at the embryonic pole each contain one lateral half of the shield, the extra embryonic fragments include only germ-ring and extra-embryonic membrane

Principal interest, however, centers on the seventeen grafts in which embryonic fragments were isolated on the yolk-sac epithelium at some distance from the host. Like the isolated germ-ring grafts described previously, twelve of these developed but failed to differentiate embryonic structures or tissues. Some of them remained as solid masses of cells, others became vesicular; a number of them contained erythrocytes or chromatophores, a characteristic already described for otherwise nondifferentiating grafts of Fundulus germ-ring.

The five remaining grafts, all of which represented extra-embryonic sixths of the blastoderm, successfully completed some histogenesis. Two small grafts, fixed at stage 31, were each constituted solely of a mass of central nervous tissue containing both cell bodies and nerve fibers. A

third graft, which formed a small vesicle external to the host's yolk-sac epithelium was hollow through most of its extent, containing only a small core of erythryocytes and melanophores; at one extremity it differentiated a clearly recognizable pectoral fin. This organ was distinctly retarded in development, resembling much more closely a stage 26 fin than the stage 31 counterpart in the host. In the two remaining cases, the differentiation of the grafts was more highly complex. One was fixed at stage 18 to 19, too early to recognize all tissues, but nervous system, chorda, somites, and gut were clearly differentiated. In the other, fixed at stage 21 to 22, there were present, in addition to these four axial structures, a contractile heart as large as that of the host and three otic vesicles. In neither of these latter two cases did the differentiated tissues exhibit the topographical relationships characteristic of either normal or induced embryos.

It is remarkable that differentiation is obtained under any conditions from extra-embryonic grafts containing only germ-ring and extra-embryonic membrane, in view of carbon-marking experiments recently performed[5] suggesting that neither the 90° or the 180° germ-ring in Fundulus contributes directly to the formation of the embryo. In the earlier germ-ring isolates on the yolk-sac epithelium only 1 out of 39 cases differentiated (retarded gut), so that the differentiation here in 5 out of 17 cases reflects a higher potency for differentiation in the larger fragments. The differentiation of three of the Fundulus fragments, however, was far more circumscribed than that in the trout, where the differentiating cases in a great majority of cases exhibited a complex variety of axial structures similar to that shown by only two cases in the present series.

The small number of successful cases in the present series and the lack of uniformity in their differentiation permits no analysis of the factors controlling the direction of differentiation in these isolates, but the data are sufficient to confirm the earlier surmise that the differences reported between Fundulus and Salmo reside within the biological material itself rather than in the method of its handling, and as yet no evidence is available demonstrating the existence of a physiological gradient field in Fundulus.

Summary.—The development of extra-embryonic sixths and quarters of the Fundulus gastrula has been studied after their implantation to the yolk-sac epithelium of host gastrulae. Twelve out of 17 such grafts developed but failed to differentiate. Of the remaining five, two differentiated only nervous tissue, another differentiated pectoral fin, a fourth, nervous system, chorda, somites, and gut; in the fifth these four axial tissues were accompanied by a contractile heart and three otic vesicles. These results differ from those of former experiments on Fundulus[4] in which only 1 out of 39 grafts of germ-ring differentiated after transplantation to the yolk-sac epithelium, and also from those of earlier experiments on Salmo[1] where sixths and quarters of the blastoderm in a great majority

of cases consistently differentiated a great variety of tissues. The interpretation is therefore confirmed that differences in the former results on Fundulus and Salmo are based on differences in the biological material rather than in the method of operation, and the existence of a physiological gradient field comparable to that postulated for the trout has not been demonstrated for Fundulus.

* Work performed at the Osborn Zoological Laboratory, Yale University, and the Department of Biology, The Johns Hopkins University, during the tenure of a John Simon Guggenheim Memorial Foundation Fellowship.

[1] Luther, W., *Arch. Entwmech.*, **135,** 359–383 (1936).
[2] Luther, W., *Biol. Zbl.*, **55,** 114–137 (1935).
[3] Luther, W., *Arch. Entwmech.*, **137,** 404–424 (1937).
[4] Oppenheimer, J. M., *J. Exptl. Zool.*, **79,** 185–212 (1938).
[5] Brummett, A. R., *Ibid.*; (in press).

ERRATA: SUBCELLULAR FRACTIONATION OF MOUSE SPLEEN RADIATION PROTECTION ACTIVITY

In the article of the above title appearing in these PROCEEDINGS **39,** 759–772 (1953), an error appears in the date in the first footnote on page 771. The correct date of presentation before the meeting of The American Physiological Society is April 7, 1953.

LEONARD J. COLE

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